Antioxidant Properties of Dry Extracts from Selected Lamiaceae Herbs as Studied by Isothermal Chemiluminescence Method

SILVIU JIPA[1,3]*, DAN ARTENIE MARIS[1], WILHELM KAPPEL[1], LAURA MONICA GORGHIU[3], CRINELA DUMITRESCU[3]

[1] INCDIE ICPE CA, 313 Splaiul Unirii, 030138, Bucharest, Romania

[2] Ovidius University, Faculty of Stomatology, 124 Mamaia Blvd., 900527, Constanta, Romania

[3] "Valachia" University of Targoviste, 18-22 Unirii Av., 130082, Targoviste, Romania

Nine commercially available plants belonging to the Lamiaceae family were investigated for the characterization of thermal stabilization of paraffin substrate. The kinetic parameters were calculated from the isothermal chemiluminescence curves. They were correlated with the total phenolic and flavonoids contents in the studied herbs The data clearly outline that the richest phenolics content was found in Rosemary. Sage and Rosemary exhibit the highest flavonoids content through all investigated plants.

Keywords: natural antioxidants, thermal stability, chemiluminescence

The Lamiaceae (Labiatae) family is one of the largest and most distinctive families, which represent the major sources of culinary, vegetable and medicine plants all over the world. Many herbs are excellent sources of phenol compounds which have been reported to show antioxidant activity [1, 2]. They include phenolic acids and flavonoids, and, particularly, the latter is a highly diverse group. Variation in their heterocyclic ring gives rise to flavonols (fig. 1), flavones (fig. 2), flavonones (fig. 3), flavan-3-ols (fig. 4) and anthocyanidins (fig. 5). Over 4,000 different naturally occurring flavonoids have been described [3]. Numerous techniques are available to evaluate the antioxidant activities of plant extracts [4, 5] such as: Fe (II) to Fe (III) reduction [6], H_2O_2 scavenging [7], DPPH [8] and ·OH [9] radicals-scavenging, etc. We chose nine commercially available plants of some species from the Limiaceae family for testing their antioxidant activity by means of isothermal chemiluminescence method. The purpose of the work is to evaluate the relationship between the observed antioxidant properties and the phenolic content of the extracts for the practical applications in food preparation, package manufacture and in the material thermal stabilization.

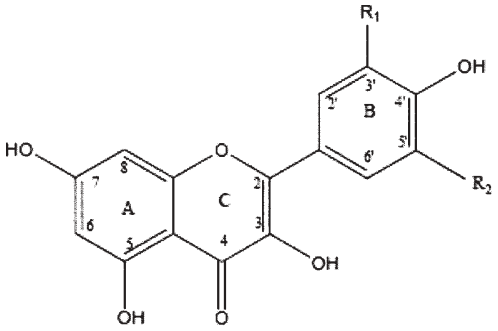

Fig. 1. Chemical structure of some flavonols

Isorhamnetin: R_1= OMe R_2 = H
Kaempferol: R_1= H R_2 = H
Myricetin: R_1= OH R_2 = OH
Quercetin: R_1= OH R_2 = H

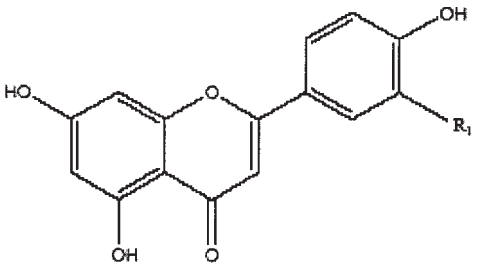

Fig. 2. Chemical structure of some flavones.
Leuteolin: R_1= OH

Experimental part

Nine plant herbs from Limiaceae (Labiatae) family were used in our study (table 1). Only samples complying with the requirements of good manufacturing practice were considered. These plants were cut up and further subjected to extraction by maceration in ethanol for 120 h at room temperature; 10 g of grounded plant were subjected to extraction with 100 mL of pure ethanol. The solution was filtered using a 0.45 μm filter. The solvent was evaporated

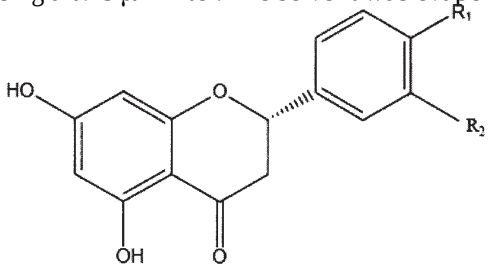

Fig. 3. Chemical structure of some flavonones

Eriodictyol: R_1= OH R_2 = OH
Hesperetin: R_1= OMe R_2 = OH
Naringenin: R_1= OH R_2 = H

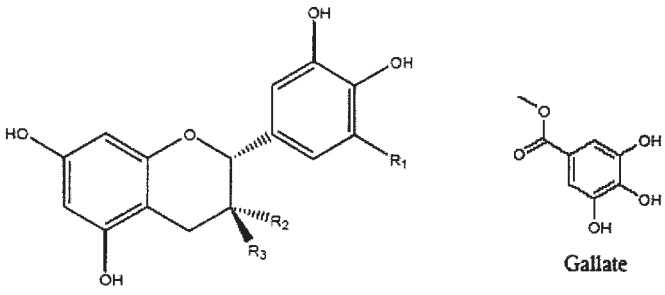

Fig. 4. Chemical structure of some flavon-3-ols (catechins and epicatechins)

(+) Catechin: R_1= H, R_2 = H, R_3 = OH,
(+) Catechin-3-gallate: R_1= OMe, R_2 = H, R_3 = gallate,
(-) Epicatechin: R_1= H, R_2 = OH, R_3 = H,
(-) Epicatechin-3-gallate: R_1= H, R_2 = gallate, R_3 = H,
(-) Epigallocatechin: R_1= OH, R_2 = OH, R_3 = H,
(-) Epigallocatechin-3-gallate R_1= OH, R_2 = gallate, R_3 = H,
(+) Gallocatechin R_1= OH, R_2 = H, R_3 = OH,
(+) Gallocatechin-3-gallate: R_1= OH, R_2 = H, R_3 = Gallate

* *email: jipasilviu@yahoo.com*

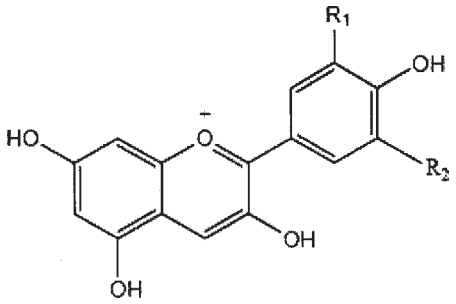

Fig. 5. Chemical structure of some anthocyanins

Cyanidin:	R_1= H	R_2 = OH
Delphinidin:	R_1= OH	R_2 = OH
Malvidin:	R_1= OMe	R_2 = H
Pelargonidin:	R_1= H	R_2 = OH
Peonidin:	R_1= OH	R_2 = OH
Petunidin:	R_1= OMe	R_2 = H

under vacuum to dryness. The extracts were stored in a dry and cool place.

The dry extracts were used for paraffin addition (0.25 % w/w).

Round aluminium trays were used to support the sample in the oven of the CL equipment. Several isothermal CL determinations were performed in air at 153^{0}C in an oxyluminograph OL-94 instrument. Details on this equipment and on measurement procedure have been previously presented [11]. This device allows the determination of the dependence of photon counts on oxidation time. From the experimental data (fig. 6) induction onset was calculated and it was taken as a reference parameter, which can be defined as the time before an accelerated oxidation change occurs, i.e., a measure of time before manifestation of oxidation.

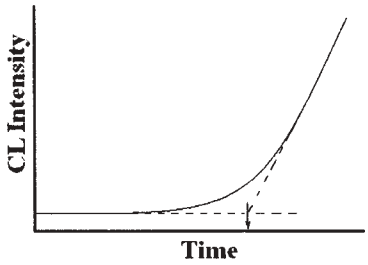

Fig. 6. Graphical method for the determination of induction time

Results and discussion

The dependencies of specific CL intensity on time for the studied extracts are presented in figure 7.

Table 2 lists the main kinetic parameters calculated from CL measurements.

Table 1
LIST OF STUDIED HERBS

Common name	Botanical name
Basil	Ocimum basilicum L.
Hyssop	Hyssopus officinalis L.
Lemon balm	Melissa officinalis L.
Levender	Lavandula angustifolia L.
Marjoram	Majorana hortensis L.
Mint	Mentha spicata L.
Mild oregano	Origanum vulgaris L.
Rosemary	Rosmarinul officinalis L.
Sage	Salvia officinalis L.

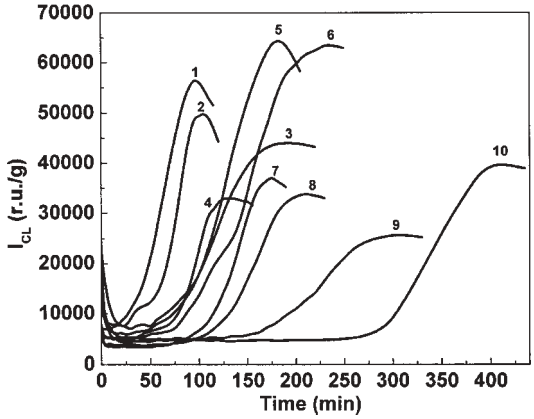

Fig. 7. Isothermal CL curves (air, at 153^{0}C) for paraffin stabilized (0.25 % w/w) with studied extracts. (1)blank, (2) Basil, (3) Lavender, (4) Mild oregano, (5) Mint, (6) Lemon balm, (7) Marjoram, (8) Hyssop, (9) Rosemary, (10) Sage

Based on length of the CL induction time, the majority of studied herbs showed significant antioxidant activity. The extracts can be classified in three groups according to their stabilization efficiency. The first group consisting of Sage, Rosemary, Hyssop and Marjoram is characterized by the longest oxidation induction time and the lowest oxidation rate. The values of these parameters demonstrate their high capacity to prevent oxidation. The second group of medium activity extracts includes Lemon balm, Mint and Mild oregano. There extracts display moderate values of oxidation induction time. The third group of extracts reveals very slight properties in the hindering of oxidation and it consists of Levender and Basil.

The fact that the maximum oxidation rate (i. e. the slope of the curves in fig. 7) is smaller than in the case of blank sample suggests that the phenolic compounds present in the extracts may be capable of chain-breaking antioxidant action during the propagation stage of oxidation.

Table 2
KINETIC PARAMETERS FOR THERMAL OXIDATION OF STABILIZED PARAFFIN (0.25 % W/W) IN AIR AT 153^{0}C

Extract	t_i (min)	$t_{1/2}$ (min)	V_{ox}^{max} (r.u./g.min)	I_{max} (r.u./g)	t_{max} (min)
Blank	5	33	982	56634	70
Basil	39	78	337	27525	130
Levender	64	110	473	44020	190
Mild oregano	70	91	769	32965	130
Mint	83	123	767	65245	175
Lemon balm	98	145	765	63600	230
Marjoram	103	132	660	37255	175
Hyssop	110	150	413	33900	210
Rosemary	179	229	517	52450	330
Sage	275	330	360	39800	405

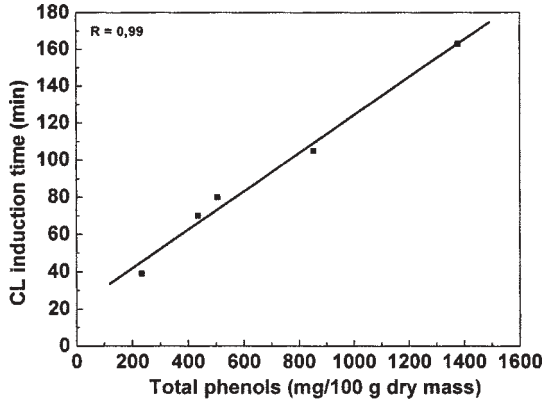

Fig. 8. Correlation between the total phenolic content and CL induction time

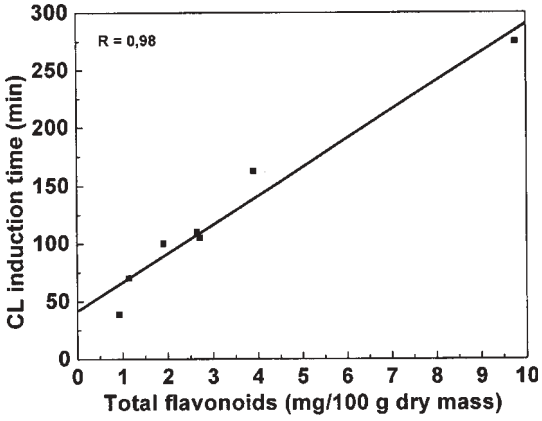

Fig. 9. Correlation between the total flavonoid content and CL induction time for selected extracts from Lamiaceae herbs

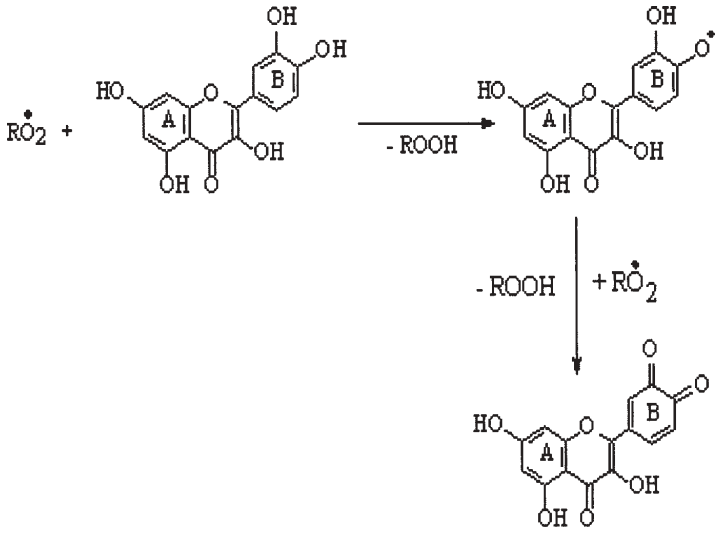

Fig. 10. Mechanism of stabilization promoted by studied antioxidative herb extracts

Table 3

SOME CITED LITERATURE DATA ON THE ANTIOXIDANT EFFICIENCY FOR THE DIFFERENT HERB EXTRACTS

Relative antioxidant efficiency of extracts	Analytical methods	References
Sage > Rosemary	Oxygen uptake	[12]
Sage > Rosemary	Gas chromatography	[13]
Rosemary > Sage >Marjoram	Peroxide value	[14]
Marjoram > Mint > Basil	Peroxide value	[15]
Mint > Oregano > Basil	Peroxide value	[16]
Sage > Rosemary > Marjoram,	-	[17]

Table 4

TOTAL PENOLIC CONTENT [19] AND TOTAL FLAVONOIDS CONTENT [20] OF SELECTED LAMIACEAE HERBS

Extract	Total phenol (mg/100 g of dry mass)	Total flavonoids (mg/100 g of dry mass)
Sage	-	9.76
Rosemary	1377	3.91
Hyssop	-	2.65
Marjoram	854	2.71
Lemon balm	-	1.91
Mint	506	-
Mild oregano	435	1.14
Basil	234	0.93

The study showed that, from 9 tested herb extracts, the Sage and Rosemary have the strongest antioxidant activities. The results on antioxidant activity obtained from herb extracts are comparable with those presented in the literature. Table 3 confirms the validity of the antioxidative efficiency order obtained by the isothermal chemiluminescence method.

The comparison is complicated by several factors. For example, the antioxidant activity varies according to the country in which the plant is grown and depends also on the type of the substrate used in the evaluation and the oxidation conditions [18].

The total phenolic and flavonoid contents in the studied herbs are presented in table 4. The data clearly outline that the richest phenolics content was found in Rosemary [19]. Sage and Rosemary have the highest flavonoids content [20].

It is interesting to observe the linear correlation between the total phenolic content (fig. 8) as well as total flavonoids content (fig. 9) and the antioxidant activity expressed by the induction oxidation time.

The correlation coefficient between these two parameters was higher than 0.9 indicating that there is a significant positive relationship between CL induction time and total phenolic and flavonoids contents of plant extracts selected in this study.

The key role of flavonoids, as scavengers of free radicals, is emphasized in several papers [21 - 24]. The mechanism through which this kind of stabilisers acts in polymer materials is shown in figure 10. Antioxidant activity is dependent on the structure of the free radical-scavenging compounds and the substituents there are presented on the ring of flavonoids [23]. Antioxidant activities are primarily attributed to the high reactivity of hydroxyl substituents. Peroxyl radicals can abstract the two hydroxyl hydrogens of β-ring producing the corresponding inactive quinones.

Conclusions

The results show that all investigated plants extracts have antioxidant effect.

The extracts with higher total phenolic and flavonoid contents were superior in antioxidant activity.

The Sage and Rosemary extracts showed remarkable degree of antioxidant activity.

The correlation coefficient from regression analysis showed a positive relationship between total phenolic ($r = 0.99$) and flavonoids ($r = 0.98$) content vs CL induction time.

The chemiluminescence technique for measuring antioxidant activity had produced results in accordance with literature findings.

References

1.RICE-EVANS, C., MILLER, N., PAGANGA, G., Radical Biology and Medicine, 20, 1996, p. 933

2.ZHENG, W., WANG, S., J. Agric. Food Chem., 49, 2001, p. 5165

3.MIDDLETON, E., KANDASWAMI, C., The Flavonoids: Advances in Research Science, 1986, ed. J., B. Harborne, Chapmann and Hall, London, p. 619

4.ANATOLOVICH, M., PRENZLER, P. D., PATSALIDES, E., McDONA, D. S., ROBARDA, K., The Analyst, 127, 2002, p. 183

5.ARMSTRONG, D., Free Radicals and Antioxidant Protocols, in D. Armstrong (Ed), Methods in Molecular Biology, **108**, Humana Press, NJ, USA

6.OYAIZU, M., Jpn. J. Nutr., 44, 1986, p. 307

7.RUCH, R. J., CHENG, S. J., KLAUNING, J. E., Carcinogen, 10, 1989, p. 1003

8.BLOIS, M. S., Nature, 181, 1958, p. 1199

9.HALLIWELL, B., BUTTERIDGE, J. M. C., ARNOMA, O. L., Anal. Biochem., 165, 1987, p. 215

10.SLINKARD, K., SINGLETON, Amer. J. Enol. Vitic., 28, 1977, 49

11.JIPA, S., ZAHARESCU, T., SETNESCU, R., SETNESCU, T., BRITES, M. J. S. SILVA, A. M. G., MARECELO-CURTO, A. J., GIGANTE, B., Polym. Int., 48, 1999, p. 414

12.BRACCO, U., LÖLIGER, J., VIRET, J. L., J. Amer. Oil Chem. Soc., 58, 1981, p. 686

13.CUVELIER, M. E., BERSET, C., RICHARD, H., Sci. Aliments, 10, 1990, p. 797

14.PALITZSCH, A., SCHULZE, H.,. LOTTER, G., STEICHELE, A., Fleischwirtschaft, 54, 1974, p. 63

15.ECONOMOU, K. D., OREOPOULUO, THOMOPOULOS, C. D., J. Amer. Oil Chem. Soc., 68, 1991, p. 109

16.YANISHLIEVA, N. V., MARINOVA, E. M., Nahrung, 39, 1995, p. 458

17.GERHARDT, U., BÖHM, Fleischwirtschaft, 60, 1980, p. 1523

18.POKORNY, J., YANISHIEVA, N., GORDON, M. (Eds), Antioxidants in Food. Practical Applications, CRC Press, 2001, p. 244

19.NINFALI, P., MEA, G., GIORGINI, S., ROCCHI, M., BACCHIOCCA, M., Brit. J. Nutrition, 93, 2005, p. 257

20.*** USDA Database for the Flavonoid Content of Selected Food, Maryland, USA, August 2006

21.WANG, H., CAO, G., PRIOR, R. L., J. Agric. Food Chem., 45, 1997, p. 304

22.SAINT-CRICQ DE DAULEJAC, PROVOST, N., VIVAS, C., VIVAS, N., J. Agric. Food Chem., 47, 1999, p. 425

23.CHENG, Z. Y., CHAN, P. T., HO, K. Y., FUNG, K. P., WANG, J., Chem. Phys. Lipids, 79, 1999, p. 157

24. BUMBAC, M., GORGHIU, L. M., DUMITRESCU, C., JIPA, S., SETNESCU, R., Mater. Plast., **42**, nr. 4, 2005, p. 313

Manuscript received. 6.08.2007