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Scavenger capacity of natural phenolics in some selected labiatae herbs

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The scavenging capacity of several labiatae plant extracts in thermal oxidation of paraffin was investigated. The stabilization activity was determined by isothermal chemiluminescence. The studied extracts produced large increase in the oxidation induction time (55 times for sage, 36 times for rosemary, 20 times for wild thyme and 14 times for oregano) in respect with neat paraffin. Other characteristics such as half time oxidation and oxidation rate are significantly improved.

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1. Introduction

The effective protection against thermal naturally-occurring antioxidants from plants has been extensively studied for the antioxidative property [1-4]. Being widely distributed throughout the plants phenolic compounds are one of the most numerous classes of substances. More than 8000 substances have been discovered so far and their number is constantly increasing [5].

Labiatae plants are rich in phenolic compounds from several classes: di- and triterpenes, simple phenolics, phenolic acids and flavonoids. From sage, more than 160 different phenolic constituents have been identified [6].

Phenolic acids were reported to be the main type of phenolic compounds in alcoholic extracts of sage, rosemary, thyme and oregano [7] (Table 1).

Table 1. Antioxidants isolated from Labiatae plants.

Species	Substances and type of substances	Ref.
Sage	Carnosic acid, carnosol, rosmanol, rosmarinic acid	[8 ÷ 10]
Rosemary	Carnosic acid, carnosol, rosmanol, rosmarinic acid	[8, 9, 11]
Wild Thyme	Carnosol, rosmarinic acid, carvacrol, thymol	[8 ÷ 10, 12]
Oregano	Derivatives of phenolic acids, flavonoids, tocopherols	[8, 9, 12, 13]

The phenolic acids in plants can be grouped into different subclasses according to specific substitution in the basic structure. They can form bonds with starch and other polysaccharides via hydrogen or covalent bonds and can create bridges and transverse linkages [14, 15]. The compounds of interest in the present study were from the following subclasses (Fig. 1): hydroxycinnamic acids (caffeic acid, chlorogenic acid, sinapic acid) and hydroxybenzoic acids (gallic acid, o-hydroxybenzoic acid, m-hydroxybenzoic acid).

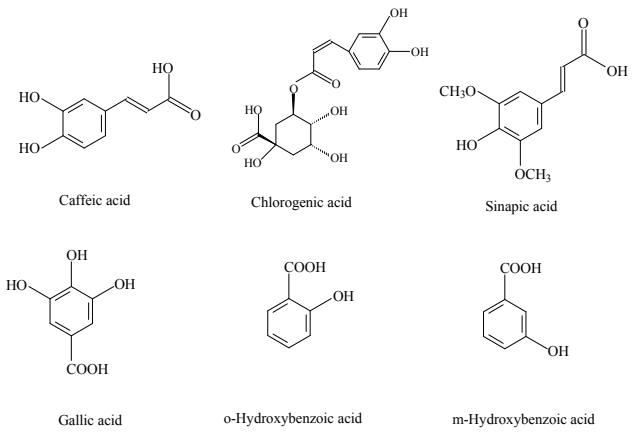

Fig. 1. Phenolic acids of interest in this study.

The purpose of the present work is to study the antioxidative effect of some phenolic acids in Labiatae plant extracts which act as free radical acceptors in thermal oxidation of an organic substrate.

2. Experimental

The analyzed dry species were: sage (Salvia Officinalis), rosemary (Rosemarinus Officinalis), wild thyme (Thymus Serpillum) and oregano (Origanum

Vulgare). The samples were ground and homogenized immediately before maceration.

The dried plant (10 g) and the extracting solvent (ethanol) were placed in an Erlenmeyer flask (250 mL); the ratio of plant material and extracting solvent was 1:10 w/v.

Maceration was performed for 120 hours at room temperature, by permanent shaking.

The liquid extract was separated from the plant material by filtration, the solvent was evaporated under vacuum, and the dry extract was gamma irradiated. The irradiated extract has been used for paraffin aditivation (0.25 wt %).

Isothermal oxyluminescence determinations were performed in air at 150°C in an oxyluminograph OL-94 instrument. Details of this equipment and of measurement procedure have been previously presented [16]. The meanings of kinetic parameters that are evaluated in this paper are presented in Fig. 2.

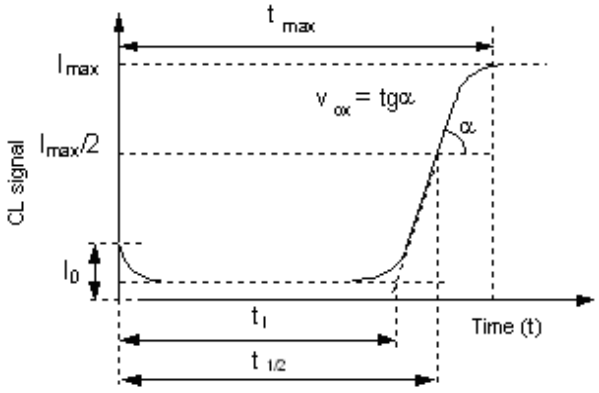

Fig. 2. The kinetic parameters from typical chemiluminogram.

All chemicals used were of analytical grade purity.

3. Results and discussions

Fig. 3 shows the chemiluminescence spectra (150°C, air) of Labiatae plant extracts in paraffin (0.25 wt %). The kinetic analysis of chemiluminescence data was performed using the following parameters: the induction time (t_i), the maximum oxidation rate (V_{ox}^{max}), the CL emission maximum intensity (I_{max}) and the time for reaching the CL emission maximum value (t_{max}) (Table 2).

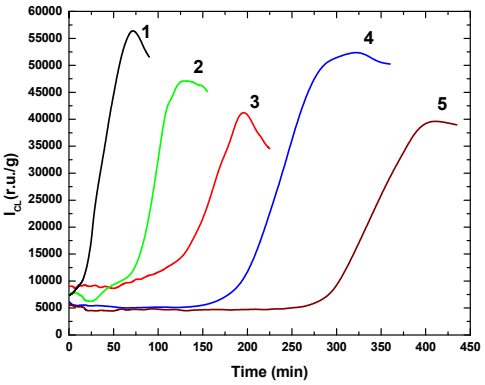

Fig. 3. CL spectra of thermal degraded paraffin (150°C, air) in the presence of different Labiatae plant extracts (0.25 wt %): (1) undoped sample; (2) oregano; (3) wild thyme; (4) rosemary; (5) sage.

Jones et al [17] pointed out that the critical hydroperoxyd concentration is reached when 50% of a substance is oxidized. Using this assumption, the time corresponding to $I = 0.5 \cdot I_{max}$ was also determined. In table 2 this $t_{1/2}$ parameter is included.

Table 2. Values of the kinetic CL parameters (150°C, air) of paraffin doped with different Labiatae plant extracts (0.25 wt %).

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
Sage	275	330	360	39800	405
Rosemary	179	229	517	52450	330
Wild thyme	100	150	380	31900	200
Oregano	70	91	769	32965	130
Butylated hydroxytoluene (BHT)	133	164	285	20100	230

As was expected, the Labiatae plant extracts inhibited paraffin degradation at 150°C by trapping the radicals formed in system. This is proved by the increased values of t_i, $t_{1/2}$ or t_{max} and by the diminution of V_{ox}^{max} and I_{max} by comparison with the blank sample. Therefore the Labiatae plant extracts are effective as peroxyl radical chain interrupters and can be regarded as potentially substitute for chemical synthetic antioxidants.

The antioxidative effectiveness of Labiatae plant extracts during the oxidation of paraffin is as follows:

sage > rosemary > wild thyme > oregano

This order of efficiency is similar to that established by Wojdylo et al [2] for the estimation of total phenolic content by using Folin – Ciocalteu calorimetric method [18].

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet quenchers as well as to ability of the phenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) [19].

Fig. 4 shows the chemiluminescence (168°C, air) of paraffin doped with different phenolic acids.

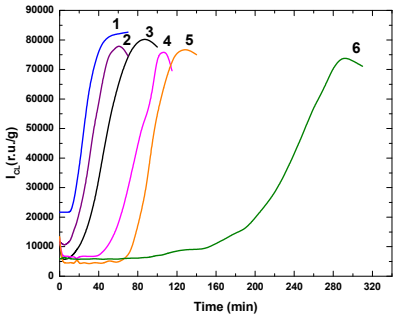

Fig. 4. CL spectra of thermal degraded paraffin (168°C, air) doped with different phenolic acids (0.25 wt %): (1) m-hydroxybenzoic; (2) hydroxybenzoic; (3) chlorogenic; (4) gallic; (5) sinapic; (6) caffeic.

All phenolic acids produced more or less pronounced retardation or inhibition of the oxidation process. However, the chemiluminograms analysis (Table 3) indicates that the phenolic acids belonging to hydroxycinnamic derivatives differ by a stronger antioxidative features from phenolic acids of group of hydroxybenzoic derivatives. The substitution of the hydroxyl groups with methoxyl groups decreases the antioxidant activity. These conclusions are in a good accordance with the studies of Vellioglu et al [20], Kim et al [21] and Rice-Evans [22].

This antioxidative assessment of phenolic acids showed that the caffeic acid could oxidation of paraffin.

Table 3. Time kinetic parameters for paraffin thermal oxidation in presence of different phenolic acids.

Phenolic acid	t_i (min)	$t_{1/2}$ (min)	t_{max} (min)
Caffeic	185	233	280
Sinapic	75	92	140
Gallic	50	74	110
Chlorogenic	23	44	100
o-Hydroxybenzoic	13	30	60
m-Hydroxybenzoic	7	22	90

The antioxidant activity of the labiatae plants is also attributed to phenolic diterpenes (Fig. 7). Among these compounds, carnosic acid is believed to posses the highest antioxidant ativity [23, 24].

Fig. 5 shows the CL spectra for the reaction of paraffin oxidation in the presence of carnosic acid in comparison with the BHT antioxidant. As can be observed, carnosic acid possesses the higher antioxidant activity. Synthetic antioxidants such as butylated hydroxytoluene (BHT) can exhibit toxic properties, for this reason nowadays existing a strong interest for naturally occurring substances.

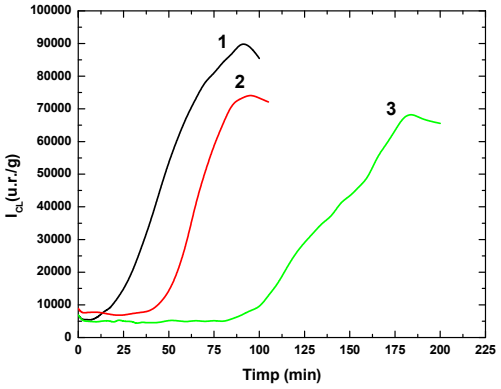

Fig. 5. CL spectra for thermal oxidation of paraffin in presence of various antioxidants (0.25 wt %): (1) without inhibitor; (2) BHT; (3) carnosic acid.

Carnosic acid has its potent antioxidant activity based on oxidation cascade (Fig. 6): after its molecule has extracted a free radical becomes carnosol. Carnosol also extracts a free radical becoming rosmanol. Rosmanol continues the free radical scavenging until galdosol is formed, and further continues the scavenging process.

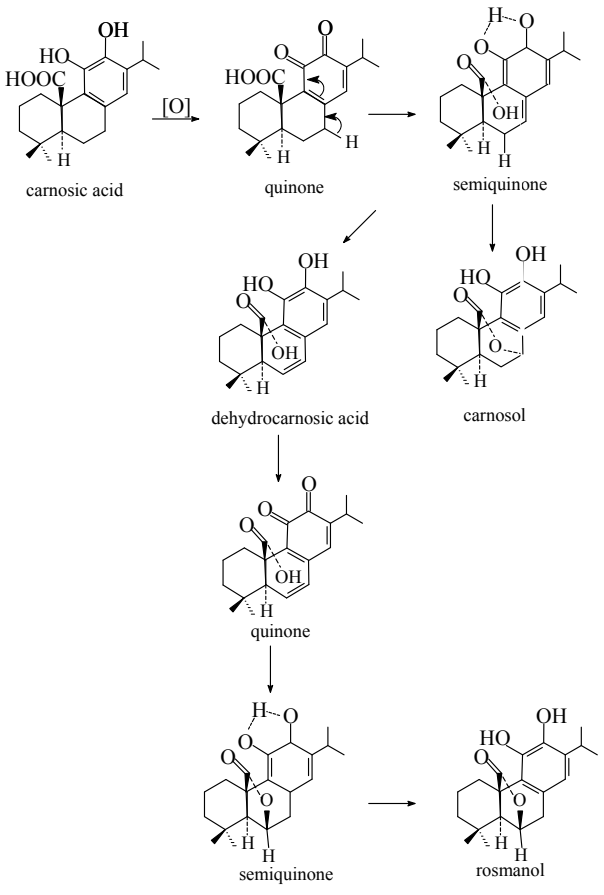

Fig. 6. Oxidation cascade reactions of carnosic acid.

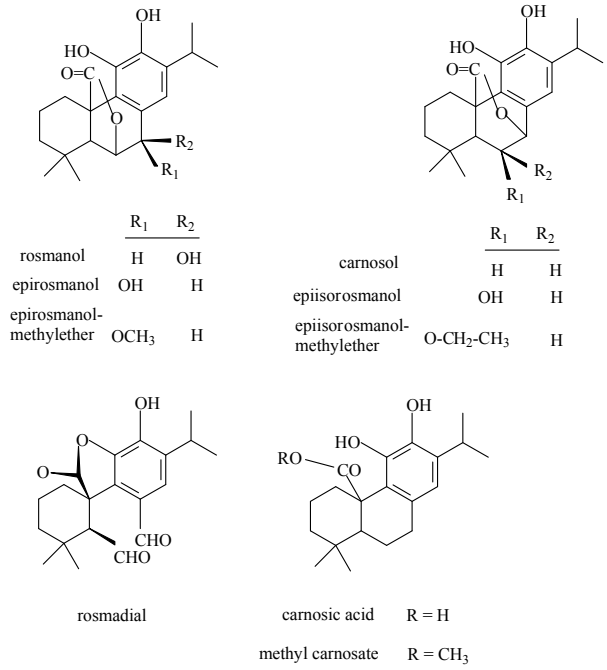

Fig. 7. Phenolic diterpenes identified from sage and rosemary extracts.

The synergistic effects of rosemary extracts with other antioxidants have been investigated and conflicting results have been reported [25, 26]. Therefore, additional data are needed to fully evaluate the synergistic effects of rosemary extracts with other natural antioxidants.

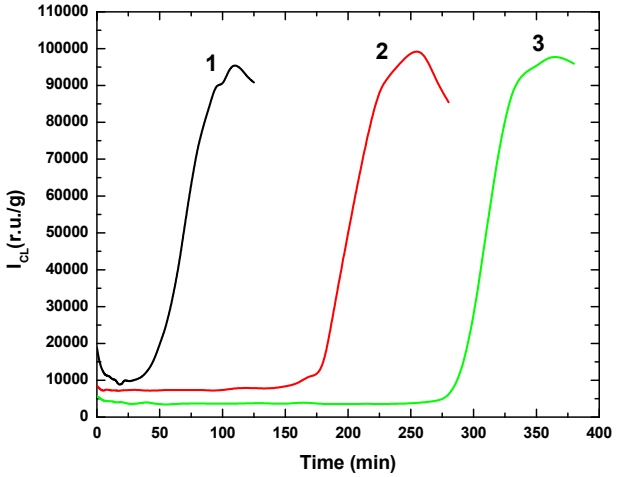

Fig. 8. CL spectra of thermal degraded paraffin containing (1) 0.25 wt % rosemary extract; (2) 0.25 wt % quercetin; (3) their mixture.

A synergistic effect between rosemary extract and quercetin has been observed (Fig. 8). This cooperative effect can be explained by regeneration mechanism between antioxidants, with the possible formation of stable intermolecular complex [27]. The cooperative factor defined as the ratio between the sum of individual effects and the similar value for the simultaneous effect has been calculated with the relationship [28]:

$$\theta = \frac{t_{1,2}}{t_1 + t_2}$$

$\theta > 1$: synergistic effect
$\theta = 1$: additivity
$\theta < 1$: antagonistic effect

where t is the induction time; In our case $\theta = 1.29$.

The antioxidative effect of Labiatae plant extracts is also based on low-molecular weight phenolic substances as flavonoids (e.g. apigenin, luteolin, naringenin etc). Several studies have shown the flavonoids to act as scavenger of peroxyl radicals [29-31]. The radicals are made inactive, according to the following equation:

$$\mathbf{Flavonoid(OH) + R\dot{O}_2 \rightarrow Flavonoid(\dot{O}) + ROOH}$$

where $\mathbf{R\dot{O}_2}$ is a peroxyl radical and $\dot{O}$ is an oxygen free radical.

4. Conclusions

The effect of natural antioxidants on the delaying of thermal degradation is beneficial by the efficient blocking of free radical oxidation. Relative to neat organic substrates, natural phenolics extracted from plants bring about significant improvement in kinetic parameters of oxidative aging. It depicts positive gain for delaying degradation under hard conditions of service for organic materials.

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