NPC Natural Product Communications

2006
Vol. 1
No. 12
1123 - 1128

Antioxidant Activity Analysis for the Selection of *Rosmarinus officinalis* L.

Juan Antonio Garbarino[a], Nicolás Troncoso[b], Pia Delpiano[b], Loreto Carvajal[b] and Alessandra Russo[c*]

[a]*Department of Chemistry, University T.F. Santa Maria, Casilla 110-V, Valparaiso, Chile*

[b]*Fundaciòn Chile, Area Agroindustrias, Av. Parque Antonio Rabat Sur 6165 Vitacura, Santiago, Chile*

[c]*Department of Biological Chemistry, Medical Chemistry and Molecular Biology, University of Catania, v.le A. Doria 6, 95125, Catania, Italy*

alrusso@unict.it; ales0303@libero.it

Received: June 23rd; Accepted: August 25th, 2006

Dedicated to the memory of Professor Ivano Morelli.

Rosmarinus officinalis L. presents a high genetic variability, which is reflected in the chemical composition of the different individuals, and consequently in its biological activity, including antioxidant capacity. The aim of the present research was to correlate the chemical composition of methanolic extracts of the dried leaves of eight rosemary accessions with their antioxidant activity for the selection of plants to optimize the use of rosemary. The eight samples examined, starting from a collection of more than 160 individuals selected by BOTANE Ltd, were cultivated at Illapel, north central Chile, using the same cultivation techniques. The free radical-scavenging capacity was tested by the ability of extracts to bleach the stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) and to inhibit superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($^{\cdot}OH$) production. The metal chelating activity was estimated by the ferrozine assay. All extracts (1-8) contained high concentrations of carnosic acid, and to a minor extent rosmarinic acid, and exhibited antioxidant activity. However, extracts 7 and 8, containing 31.7 and 26.1% of carnosic acid, respectively, have shown a higher biological effect, confirming that the antioxidant activity of *R. officinalis* leaves is primarily related to this phenolic diterpene and suggesting that the measure of antioxidant activity could be considered a good method in the selection of this plant for its optimization. Interestingly, our experimental evidence also suggests that air pollution negatively influences the carnosic acid content. In fact, samples 3 and 4, with a low carnosic acid content, originated from a highly polluted metropolitan area of Santiago city.

Keywords: *Rosmarinus officinalis* L., leaf extract, carnosic acid, free radicals, antioxidant activity.

There is abundant evidence that reactive oxygen and nitrogen species (ROS and RNS) are implicated in several physiological processes, such as in host defence against invading pathogens and signal transduction. An overproduction of such reactive species, however plays a major role in several pathophysiological conditions. The ROS and RNS formed may cause cellular and subcellular damage by peroxidation of membrane lipids, by denaturing cellular proteins and by the breaking of DNA strands, disrupting cellular functions [1]. Lipid oxidation may also reduce the flavor and nutritive value of fats, oils and lipid-containing products. Unsaturated fatty acids are sensitive to oxidation because of their chemical structure, and protein cross-linking, denaturation, polypeptide chain scission, enzyme inactivation and amino acid destruction in the presence of oxidizing lipids have been reported [1]. In this regard, many nutritive and non-nutritive phytochemicals, containing principally polyphenolic compounds and with diverse biological properties, have shown promising responses for the prevention and/or intervention of all diseases in which oxidative stress plays a key role [2]. A large number of polyphenolic compounds with antioxidant activity have been identified in the Labiatae plant

Rosmarinus officinalis L., including phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, 7-methylepirosmanol, and methyl carnosate. In addition, several flavonoids, such as genkwanin, hispidulin 7-*O*-glucoside, cirsimaritin, luteolin, and isoscutellarein 7-*O*-glucoside, are found in Labiatae plants; the phenolic compounds rosmarinic and caffeic acids are also present [3-8]. *R. officinalis* (rosemary) is a typical Mediterranean species, but now is cultivated all over the world. Usually the plant is clonally propagated because of the poor germinability of its seeds and the genetic diversity of the seedlings [9]. *R. officinalis* presents, in fact, a high genetic variability, which is reflected in the chemical composition of the different individuals, and probably in its biological activity.

This plant is used as a spice and folk medicine around the world, as well as in cosmetics. The leaves are used in the preparation of alcoholic beverages, herbal soft drinks and in food preservation. In medicine, the extract is receiving increasing attention due to its antimicrobial, anti-inflammatory and antioxidative constituents [9].

The antioxidant properties of rosemary have been well documented, and there are several reports that have established carnosic acid as the major phenolic diterpenoid present in rosemary leaves with antioxidant activity [10]. Recently this phenolic compound has attracted wide interest as a potential therapeutic agent against several diseases, and research was started to investigate new biological activities. Studies showed that it has chemopreventive, anti-neoplastic [11-13] and radioprotective-antimutagenic [14] effects.

Therefore, the aim of the present research was to correlate the chemical composition of the methanolic extracts of leaves from eight different rosemary accessions, cultivated in Chile using the same cultivation techniques, with their antioxidant activity for the selection of *R. officinalis*, with the view to optimize its use.

Table 1: Origin of plant materials.

Accessions	Country
1	Santiago (Illapel), Chile
2	Santiago (Las Condes), Chile
3	Santiago (Renca), Chile
4	Santiago (Renca), Chile
5	Goodwood, Canada
6	Goodwood, Canada
7	Goodwood, Canada
8	Goodwood, Canada

The plant accessions were collected throughout Chile and other countries (Table 1), and were clonally propagated in order to maintain genetic uniformity. The fresh rosemary samples were dried, and double extraction for 159 hours was conducted for total extraction of carnosic and rosmarinic acids, which was confirmed by chromatographic analysis (data not shown). The yields of extraction of the samples are given in Table 2.

Table 2: Yield of extraction.

	Dry leaves g	First Extraction g	Second Extraction g	Total extract g	Yield %
1	58.0	5.4	4.8	10.2	17.6
2	50.3	5.3	3.1	8.4	16.7
3	52.1	5.6	5.0	10.6	20.3
4	51.7	4.9	3.3	8.2	15.9
5	51.3	3.7	3.5	7.2	14.0
6	50.1	5.0	4.0	9.0	18.0
7	55.4	6.6	6.1	12.7	22.9
8	65.9	6.1	5.9	12.0	18.2

Table 3: Content of carnosic acid and rosmarinic acid of methanol extracts of leaves from different accessions of *Rosmarinus officinalis*.

Extracts	Carnosic acid (%)	Rosmarinic acid (%)
1	13.8±2.6	1.14±0.11
2	19.3±2.2	0.46±0.13
3	14.3±2.6	0.79±0.08
4	10.8±3.2	1.17±0.11
5	11.7±3.0	0.84±0.08
6	12.8±2.8	1.32±0.15
7	31.7±4.9	0.41±0.14
8	26.1±3.1	0.43±0.08

n=6

The biological effects exhibited by these rosemary samples, under our experimental conditions, could be related to an overall effect of the phenolic compounds present in the extracts, but carnosic acid, as previously reported [10], seems to play a key role in the antioxidant activity. All extracts (1-8) containing high concentrations of carnosic acid (Table 3), and, to a minor extent, rosmarinic acid, exhibited antioxidant properties (Tables 4, 5). However, extracts 7 and 8 containing 31.7 and 26.1% of carnosic acid, respectively (Table 3), have shown a higher antioxidant capacity. In fact, the results, summarized in Table 4, showed that all extracts exhibited DPPH free radical scavenging activity, but samples 7 and 8 exhibited higher capacity with IC_{50} values (concentration that inhibited radicals by 50%) of 9.2 and 8.6%, respectively. As DPPH is a synthetic radical, we also investigated the superoxide anion scavenging capacity of these extracts using the method of Paoletti [15], which excludes the Fenton-type reaction and the xanthine/xanthine oxidase system. Also in this assay, samples 7 and 8 showed a major superoxide scavenging effect (Table 4).

Table 4: Scavenger effect of leaf methanol extracts of different accessions of *Rosmarinus officinalis* on DPPH stable radical and superoxide anion.

	DPPH Test	Superoxide radicals
	[a]IC_{50} (μg/mL)	
1	14.8±1.1	18.3±0.9
2	16.1±1.1	22.9±0.8
3	16.8±0.9	24.3±0.7
4	17.9±1.2	24.0±0.4
5	19.4±1.5	26.9±1.1
6	15.8±0.7	20.8±1.1
7	9.2±1.2	13.0±1.1
8	8.6±0.5	12.0±0.8
[b]**Trolox**	95±1.4	-
[c]**SOD**	-	87±3.4

[a]concentration that inhibited radicals by 50%. Values represent the mean ± SD of three experiments, performed in duplicate.
[b]Trolox (50 μM) and [c]superoxide dismutase (SOD) (80 mU/mL) were used as a standard; the results are expressed as % of inhibition.

Although both $O_2^{\cdot-}$ and H_2O_2 are potentially cytotoxic, most of the oxidative damage in biological systems is caused by the $^{\cdot}OH$ radical, which is generated by the reaction between $O_2^{\cdot-}$ and H_2O_2 in the presence of transition metal ions [1]. Based on the data obtained from this study, rosemary extract might also be able to modulate hydroxyl radical formation, acting as a direct scavenger and chelating ion. In fact, all extracts examined exhibited protection against DNA strand scission induced by $^{\cdot}OH$ radicals, generated by UV-photolysis of H_2O_2 (Table 5), and showed metal chelating activity capturing ferrous ions before ferrozine (Table 5). Also in these assays, samples 7 and 8 exhibited a higher effect (Table 5).

Table 5: Effect of methanol extracts of leaves from different accessions of *Rosmarinus officinalis* (100 μg/mL) on DNA cleavage induced by the photolysis of H_2O_2 and metal chelating activity.

	UD of supercoiled DNA	Ferrozine assay
	[a]% of native DNA	[b]IC_{50} (μg/mL)
scDNA	100	
1	46±1.4*	83.2±1.4
2	51±1.7*	89.4±1.8
3	53±2.1*	94.5±0.9
4	50±1.9*	96.7±1.2
5	55±1.8*	106.3±1.5
6	45±0.9*	86.3±0.7
7	83±1.6*	62.5±1.2
8	81±1.5*	57.5±0.8
[c]**DTPA**	-	77±2.7

[a]The hydroxyl radicals generated by the photolysis of H_2O_2 reduced the supercoiled DNA (SCDNA).
[b]concentration that inhibited the ferrozine-Fe^{2+} formation by 50%. [c]DTPA (5 μM) was used as a standard; the results are expressed as % of inhibition.
Values represent the mean ± SD of three experiments, performed in duplicate. *significant vs. supercoiled DNA (p<0.001).

Interestingly, our results, similar to other works reporting that environmental elements affect carnosic acid concentrations [16], also reveal a correlation between carnosic acid concentration and air pollution. In fact, samples 3 and 4, with low carnosic acid contents, 14.3 and 10.8%, respectively, correlating with a low antioxidant activity, originated from a heavily polluted metropolitan area of Santiago city, which ranks as one of the most polluted cities in the world. The main ambient pollutants of concern include carbon monoxide (CO), nitrogen oxides (NO), sulphur dioxide (SO_2), tropospheric ozone (O_3), and particulate matter (PM) [16-18].

In summary, our results suggest that the measure of antioxidant activity could be considered a good method for the selection of this plant for its optimization. Interestingly, our experimental evidence also suggests that air pollution negatively influences the carnosic acid content, justifying further studies to explain the mechanisms involved in this effect.

Experimental

Materials: Rosmarinic acid and carnosic acid, used as standards, were obtained from Addipharma and Sigma, respectively. The water used was Milli-Q quality, methanol and acetonitrile (MeCN) were from Merck and HPLC grade. Inorganic reagents from Merck were PA quality.

1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), diethylenetriaminepentaacetic acid (DTPA), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) and pBR322 plasmid DNA were obtained from Sigma Aldrich Co (St. Louis, USA); β-nicotinamide-adenine dinucleotide (NADH) was obtained from Boehringer Mannheim GmbH (Germany). All other chemicals were purchased from GIBCO BRL Life Technologies (Grand Island, NY, USA).

Plant materials: Plant accessions were kindly borrowed from the special collection of more than 160 individuals used by BOTANE Ltd. to establish its crops and were collected throughout Chile and other countries (Table 1). Accessions were clonally propagated in order to maintain genetic uniformity. Each accession was propagated and established in a greenhouse and then transplanted to square-shaped blocks. Accessions were planted in November 2001 at Illapel, in north central Chile, about 250 Km north of Santiago (31°43'S; 71°07'W; 391 m.a.s.l.). Sampling was performed in a random pattern in the square shaped blocks and on freshly grown material.

Sample preparation: The fresh rosemary samples were dried at 40◦C in a forced air circulation oven (MemmertULM500). Leaves were manually separated and ground in a vertical hammer mill (Peruzzo Milly model 35.010) at 12,000 rpm and 0.8 mm mesh. Sample humidity was determined employing a Sartorius MA30 infrared system. Samples were mixed with 500 mL of methanol and stirred for 15 h at 20 °C in the dark. After stirring and filtering under vacuum, the filtrate was evaporated to dryness in a Rotavapor. The samples were extracted again for 144 hours, as previously described, and the filtrate was evaporated to dryness in a Rotavapor. Double extraction for 159 hours was conducted for total extraction of carnosic and rosmarinic acids, which was confirmed by chromatographic analysis. The extraction yields from the rosemary samples are given in Table 2.

Chromatographic conditions: A binary MeCN-H_2O acidified gradient was used for elution, as previously reported [19]. Two different procedures were developed. Method I, for simultaneous resolution of the three compounds of interest (CA, C, RA), the solvents A and B were MeCN–H_2O–H_3PO_4 (65.1%:34.9%:0.02%) and MeCN–H_2O–H_3PO_4 (22%:78%:0.25%), respectively. At a flow of 1.5 mL/min, the eluent consisted of 100% B during the initial 2 min, then the percentage of solvent A was increased to 100% at 2.1 min and remained at this level for the next 6 min. At 8.1 min the percentage of solvent B was again increased to 100%, where it remained for the last 2 min of the run time. With this method, the retention times were for RA *t*r = 2.7 min, for C *t*r = 5.7 min, and for CA *t*r = 6.6 min. This method requires sample extraction with methanol: water (2:1) in order to extract all lipo-soluble and hydro-soluble antioxidants.

Method II: This chromatographic procedure is isocratic with solvent A as eluent for 6 min. The retention times were for C *t*r = 1.8 min, and for CA *t*r = 2.5 min. With this procedure, hydro-soluble compounds are not resolved, so sample extraction was simply performed with methanol. The detection wavelengths selected to quantify carnosic acid and rosmarinic acid were 230 and 330 nm, respectively, in order to avoid mobile phase absorption.

Antioxidant activity

Quenching of DPPH: Since the DPPH test can accommodate a large number of samples in a short period and is sensitive enough to detect natural compounds at low concentrations, it was used in the present study for a primary screening of the methanolic extracts of *R. officinalis* free radical–scavenging activity. The assay provides information on the reactivity of test compounds with a stable free radical. Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. The reaction mixture contained 86 μM DPPH, and different concentrations of the extracts (5-100 μg/mL) in 1 mL of ethanol. After 10 min at room temperature, the absorbance at λ = 517 nm was recorded [20]. Trolox (50 M), a water-soluble derivative of vitamin E, was used as a standard. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

Scavenger effect on superoxide anion: Superoxide anion was generated *in vitro* during the autoxidation of β-mercapto-ethanol, as described by Paoletti et al. [15]. The assay mixture contained, in a total volume of 1 mL, 100 mM triethanolamine-diethanolamine buffer, pH 7.4, 3 mM NADH, 25 mM/12.5 mM EDTA/$MnCl_2$, 10 mM β-mercapto-ethanol; some samples contained methanolic extracts of the samples examined of *R. officinalis*, at different concentrations (5-100 μg/mL). After 20 min incubation at 25°C, the decrease in absorbance was measured at λ = 340 nm. Superoxide dismutase (SOD) (80 mU/mL) was used as a standard. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

DNA cleavage induced by hydrogen peroxide UV-photolysis: The experiments were performed as previously reported [21], in a volume of 20 μL containing 33 μM in bp of pBR322 plasmid DNA in 5 mM phosphate saline buffer (pH 7.4), and the extracts. Immediately prior to irradiating the samples with UV light, H_2O_2 was added to a final concentration of 2.5 mM. The reaction volumes were held in caps of polyethylene microcentrifuge tubes, placed directly on the surface of a transilluminator (8000 μW cm^{-1}) at 300 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 4.5 μL of a mixture containing 0.25% bromophenol blue, 0.25% xylen cyanol FF, and 30% glycerol were added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris-borate buffer

(45 mM Tris-borate, 1 mM EDTA). Untreated pBR322 plasmid was included as a control in each run of gel electrophoresis, conducted at 1.5 V/cm for 15 hours. Gel was stained in ethidium bromide (1 μg/mL; 30 min), and photographed on Polaroid-Type 667 positive land film. The intensity of each scDNA band was quantified by means of densitometry.

Metal chelating activity: The chelating of ferrous ions by the methanolic extracts from the 8 samples examined of *R. officinalis* were estimated by the ferrozine assay [22]. Briefly, the extracts (5-200 μg/mL) were added to a solution of 0.15 mM $FeSO_4$. The reaction was initiated by the addition of 0.5 mM ferrozine and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. DTPA (5 μM) was used as a standard. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

Statistical analysis: Statistical analyses were performed using the statistical software package SYSTAT, version 9 (Systat Inc., Evanston IL, USA).

Acknowledgements - The authors gratefully thank CORFO for its financial support through the FDI AT-11 grant and Dr C. Wright for proof reading the manuscript.

References

[1] Halliwell B. (**1994**) Free radicals, antioxidants and human disease: curiosity, cause of consequence? *Lancet*, ***344***, 721-724.

[2] Dryden GW, Song M, McClain C. (**2006**) Polyphenols and gastrointestinal diseases. *Current Opinion in Gastroenterology*, ***22***, 165-170.

[3] Aruoma OI, Halliwell B, Aeschbach R, Loligers J. (**1992**) Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. *Xenobiotica*, ***22***, 257-268.

[4] Munne´-Bosch S, Schwarz K, Alegre L. (**1999**) Enhanced formation of R-tocopherol and highly oxidized abietane diterpenes in water stressed rosemary plants. *Plant Physiology*, ***121***, 1047-1052.

[5] Cuvelier ME, Richard H, Berset C. (**1996**) Antioxidative activity and phenolic composition of pilot plant extracts of sage and rosemary. *Journal of American Oil Chemistry Society*, ***73***, 645-652.

[6] Del Ban˜o M J, Lorente J, Castillo J, Benavente-Garcı´a O, Marı´n MP, Del Rio JA, Ortun˜o, A, Ibarra I. (**2004**) Flavonoid distribution during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis*. Postulation of a biosynthetic pathway. *Journal of Agricultural Food and Chemistry*, ***52***, 4987-4992.

[7] Lamaison JL, Petitjean-Freytet C, Carnat A. (**1991**) Medicinal Lamiaceae with antioxidant properties, a potential source of rosmarinic acid. *Pharmaceutica Acta Helvetica*, ***66***, 185-188.

[8] Cuvelier ME, Bondety V, Berset C. (**2000**) Behaviour of phenolic antioxidants in a partioned medium: structure-activity relationship. *Journal of American Oil Chemistry Society*, ***77***, 819-823.

[9] Flamini G, Cioni PL, Morelli I, Macchia M, Ceccarini L. (**2002**) Main agronomic-productive characteristics of two ecotypes of *Rosmarinus officinalis* L. and chemical composition of their essential oils. *Journal of Agricultural and Food Chemistry*, ***50***, 3512-3517.

[10] Backleh M, Leupold G, Parlar H. (**2003**) Rapid quantitative enrichment of carnosic acid from rosemary (*Rosmarinus officinalis* L.) by isoelectric focused adsorptive bubble chromatography. *Journal of Agricultural and Food Chemistry*, ***51***, 1297-1301.

[11] Offord EA, Gautier JC, Avanti O, Scaletta C, Runge F, Kramer K, Applegate LA. (**2002**) Photoprotective potential of lycopene, β-carotene, vitamin E, vitamin C and carnosic acid in UVA-irradiated human skin fibroblasts. *Free Radical Biological and Medicine*, ***32***, 1293-1303.

[12] Sharabani H, Izumchenko E, Wang Q, Kreinin R, Steiner M, Barvish Z, Kafka M, Sharoni Y, Levy J, Uskokovic M, Studzinski GP, Danilenko M. (**2006**) Cooperative antitumor effects of vitamin D3 derivatives and rosemary preparations in a mouse model of myeloid leukemia. *International Journal of Cancer*, ***118***, 3012-3021.

[13] Huang MT, Ho CT, Wang ZY, Ferraro T, Lou YR, Stauber K., Ma W, Georgiadis C, Laskin JD, Conney AH. (**1994**) Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Research*, ***54***, 701–708.

[14] Del Bano MJ, Castillo J, Benavente-Garcia O, Lorente J, Martin-Gil R, Acevedo C, Alcaraz M. (**2006**) Radioprotective-antimutagenic effects of rosemary phenolics against chromosomal damage induced in human lymphocytes by gamma-rays. *Journal of Agricultural and Food Chemistry*, ***54***, 2064-2068.

[15] Paoletti F, Aldinucci D, Mocalli A, Caparrini A. (**1986**) A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. *Analytical Biochemistry*, ***154***, 536-541.

[16] Wellwood CR, Cole RA. (**2004**) Relevance of carnosic acid concentrations to the selection of rosemary, *Rosmarinus officinalis* (L.), accessions for optimization of antioxidant yield. *Journal of Agricultural and Food Chemistry*, ***52***, 6101-6107.

[17] Gil L, Martinez V, Riquelme R, Ancic P, Gonzalez G, Rodriguez L, Adonis M. (**2003**) Occupational and environmental levels of mutagenic PAHs and respirable particulate matter associated with diesel exhaust in Santiago, Chile. *Journal of Occupational Environmental Medicine*, ***45***, 984-992.

[18] Bell ML, Davis DL, Gouveia N, Borja-Aburto VH, Cifuentes LA. (**2006**) The avoidable health effects of air pollution in three Latin American cities: Santiago, Sao Paulo, and Mexico City. *Environmental Research*, ***100***, 431-440.

[19] Troncoso N, Sierra H, Carvajal L, Delpiano P, Gunther G. (**2005**) Fast high performance liquid chromatography and ultraviolet visible quantification of principal phenolic antioxidants in fresh rosemary *Journal of Chromatography A*, ***1100***, 20-25.

[20] Bonina F, Saija A, Tomaino A, Lo Cascio R, Rapisarda P, Dederen JC. (**1998**) In *vitro* antioxidant activity and *in vivo* photoprotective effect of a red orange extract. *International Journal of Cosmetic Sciences*, ***20***, 331-342.

[21] Russo A, Cardile V, Lombardo L, Vanella L, Vanella A, Garbarino JA. (**2005**) Antioxidant activity and antiproliferative action of methanolic extract of *Geum quellyon* Sweet roots in human tumor cell lines. *Journal of Ethnopharmacology*, ***100***, 323-332.

[22] Dinis TC, Madeira VM, Almeida LM. (**1994**) Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, ***315***, 161-169.