ANTIOXIDANT AND RADIOPROTECTIVE FEATURES OF ROSEMARY (ROSMARINUS OFFICINALIS) EXTRACTS

MARIA MARIŞ[1], DAN A. MARIŞ[1], SILVIU JIPA[2,3], TRAIAN ZAHARESCU[2], ECATERINA TĂNASE[4], LAURA MONICA GORGHIU[3], MARIUS MARIŞ[5]

[1]*Dentistry Department, Faculty of Dental Medicine, "Ovidius" University of Constanţa, 124 Mamaia Blvd, 900527, Constanţa, Romania*
[2]*INCDIE ICPE CA, Department of Radiation Processing, 313 Splaiul Unirii, P. O. Box 149, 030138 Bucharest, România*
[3]*Sciences Department, Faculty of Science and Arts, Valahia University of Targoviste, 18-20 Unirii Blvd, 130082, Târgovişte, Romania*
[4]*Veterinary Health and Food Safety Department Constanţa, 78 Mangaliei Street, 900111, Constanţa, Romania*
[5] *Faculty of Dental Medicine,"Carol Davila" University of Bucharest, 19 Calea Plevnei Street, 010232, Bucharest,Romania*

Abstract. *Numerous studies have examined the radioprotective effects of antioxidant compounds, generally known like free radical scavengers, which protect cells from free radical damage. Various plant extracts have been investigated to evaluate their radioprotective effects.*

The present study deals with the radioprotective activity of ethanolic extract prepared from Rosmarinus officinalis in terms of antioxidative activity and on Swiss albino mice post irradiation morbidness and death rate, respectively. Effects of 0.1 and 0.2/Kg body wt. of Rosemary extract on radiation – induced morbidity and mortality in mice exposed to 10Gy of gamma radiation were studied for the characterization of high efficiency protection exhibited by Rosemary extract.

Keywords: *Rosemary, chemoluminescence, antioxidative activity, ionizing radiation, morbidity, mortality*

1. Introduction

Various initiators of carcinogenesis, for instance gamma radiation, act via generation of oxygen free radicals. These reactive oxygen species, such as hydroxyl ($HO^{\bullet}$) and peroxyl radicals ($RO_2^{\bullet}$) as well as the superoxide anions ($O_2^{\bullet -}$) are produced as a result of radiolytic reactions in living systems. The radical species cause oxidative injury by initiating chain reaction that denature proteins, oxidize lipids, fragment DNA and ultimately participate in cell death and cancer.

A number of commonly used plants have been identified as possessing anticancer activities. These include members of various families such as: Lamiaceae (basil, rosemary), Zingiberaceae (tumeric), Umbelliferae (dill, parsley) [1-11] etc. These plants contain several phytochemicals, which possess strong antioxidant activities. The antioxidants may prevent and cure cancer by protecting the cells from damage caused by free radicals chain reactions.

The radioprotectors are chemical compounds that have the ability to reduce the effects of ioniying radiation on normal tissues.

In the present study an attempt has been made to investigate the radioprotective effect of ethanoic extract prepared from Rosmarinus Officinalis in terms of antioxidant activity and the tests of Swiss albino mice post irradiation morbidness and death rate, respectively.

2. Experimental

The dried Rosemary plant (10 g) and the extracting solvent (ethanol) were placed in an Erlenmeyer flask (250mL); 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, and the solvent was evaporated under vacuum. The solid extract has been used for paraffin modification (0.25 wt %). Similarly, parrafin samples of extracts from echinacea (Echinacea angustifolia), tumeric (Curcuma longa), basil (Ocinum basilicum), parsley (Petroselium crispum), green tea (Camellia sinensis) and dill (Anethum graveolens) have been obtained.

Isothermal chemiluminescence measurements were performed in air at 160^0C by a Lumipol-3 instrument. The meanings of kinetic that are evaluated in this paper are presented in Figure 1 and Table 1.

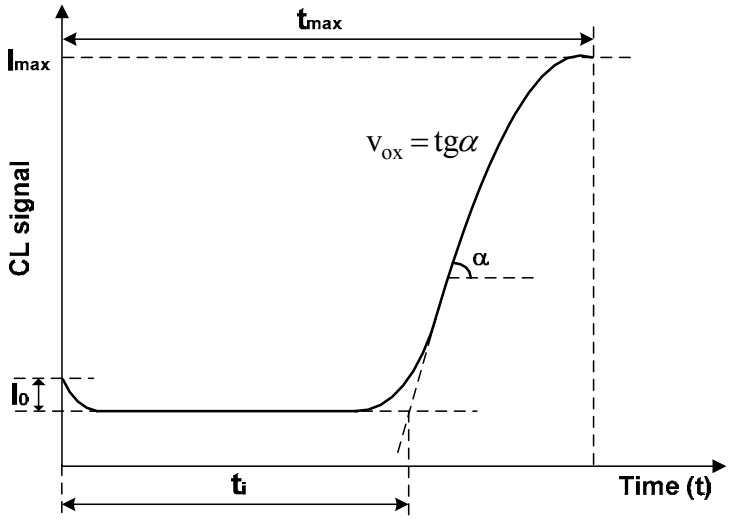

Figure 1. The kinetic parameters from typical chemiluminogram.

Table 1. CL parameters and their meaning.

Parameter	Significance
$\mathbf{t_i}$	Oxidation induction time; the time during which the CL emission intensity is stationary due to the inhibition of the oxidation by the antioxidants
$\mathbf{t_{max}}$	The time when CL intensity reaches the maximum value; it corresponds to the complete oxidation of the substrate
$\mathbf{v_{ox}}$	Oxidation rate; it is determined as the slope of the CL curve on ascending part
$\mathbf{I_{max}}$	CL maximum intensity
$\mathbf{I_o}$	CL initial intensity

The radioprotective capability of the Rosemary extract was studied on a batch of 60 Swiss albino mice. All the mice were adults, ages of over 15 weeks, both genders, weight ranging between 20 and 30 grams each. The location chosen for the experiment provided a constant environmental temperature of 21 ± 2^0C, a humidity of 50%±10%, an atmospheric pressure of 759±4 mm Hg and natural lighting. For a period of four days the mice were given tetracycline to prevent the infection.

The animals were divided into three experimental batches, each equal in number. The mice batches were kept in distinct conteiners, marked for identification.

For seven days the experimental batches were subject to a food diet in the following way:

- Batch 1: was given a standard diet;
- Batch 2: the daily diet included and average Rosemary extract dose of 0.1 g / Kg body wt.;
- Batch 3: the daily diet included and average Rosemary extract dose of 0.2 g / Kg body wt.

After seven days, the mice batches were irradiated with ^{137}Cs γ radiationst dose of 10 Gy using a GAMATOR M-38-2 source. Then, the batches were monitored to observe the occurrence of morbidity and death rate.

3. Results and discussion

Figure 2 shows the isothermal chemiluminescence curves (160^0C, air) of the paraffin stabilized with various plant extracts which prove radioprotection effect. The antioxidant efficiency in stabilizing an organic substrate is given by the increased value of the induction time in comparison with the unprotected sample (table 2). The longer is the induction taime, the stronger is the antioxidant activity.

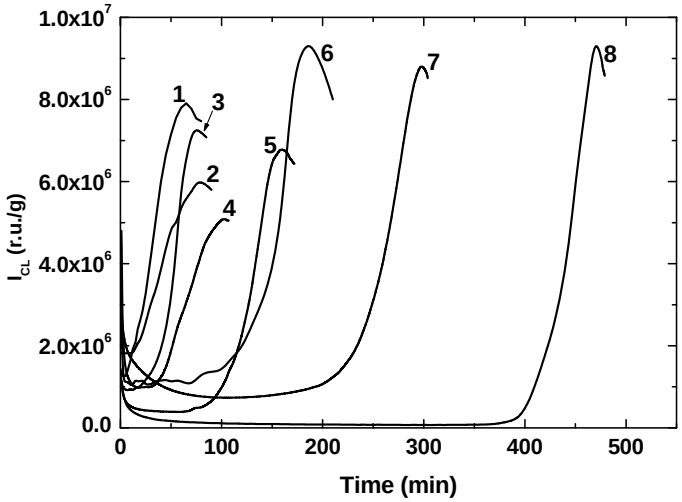

Figure 2. Isothermal CL curves (160^0C, air) for paraffin stabilized (0.25% wt%) with various plant extracts which prove a radioprotection effect: (1) Blank; (2) Echinacea; (3) Tumeric; (4) Basil; (5) Parsley; (6) Green tea; (7) Dill; (8) Rosemary

Table 2. The kinetic time parameters (CL) for paraffin doped with various radioprotective plants extracts

Extract	t_i (min)	t_{max} (min)
Blank	10	65
Echinacea (Echinacea angustifolia)	14	80
Tumeric (Curcuma longa)	42	75
Basil (Ocimum basilicum)	45	103
Parsley (Petroselinum crispum)	111	160
Green tea (Camellia sinensis)	145	186
Dill (Anethum graveolens)	239	299
Rosemary (Rosmarinus officinalis)	422	470

The analysis of the data in table 2 clearly shows that the Rosemary extract has the highest antioxidant activity. However, the antioxidant activity of Rosemary extract depends on geographical origin, climatic conditions, soil composition, cultivation practices, harvesting time, extracting method etc (Fig. 3).

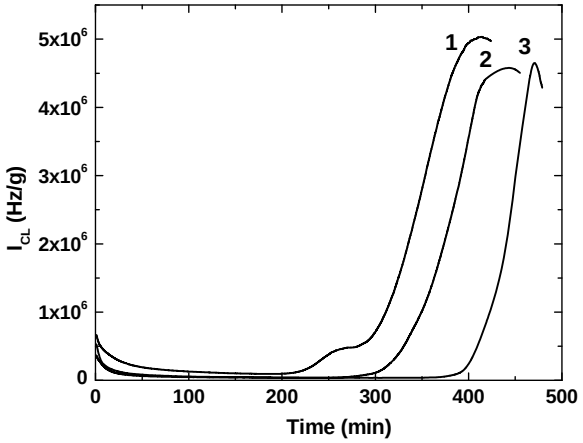

Figure 3. Chemiluminescence spectra (air, 160°C) of paraffin stabilized (0.25 wt %) with Rosemary extracts from different regions: (1) Brăila; (2) Ploieşti; (3) Târgovişte

Aruoma et co. [12] showed that the antioxidant activity of the Rosemary extract is due, mainly (over 90%) to some phenolic diterpenes such as carnosic acid, carnosol, rosmanol, isorosmanol, rosmadial, epirrosmanol, rosmaridiphenol, rosmariquinone etc (Figure 4). Most of these diterpenes act as oxidative chain breakers.

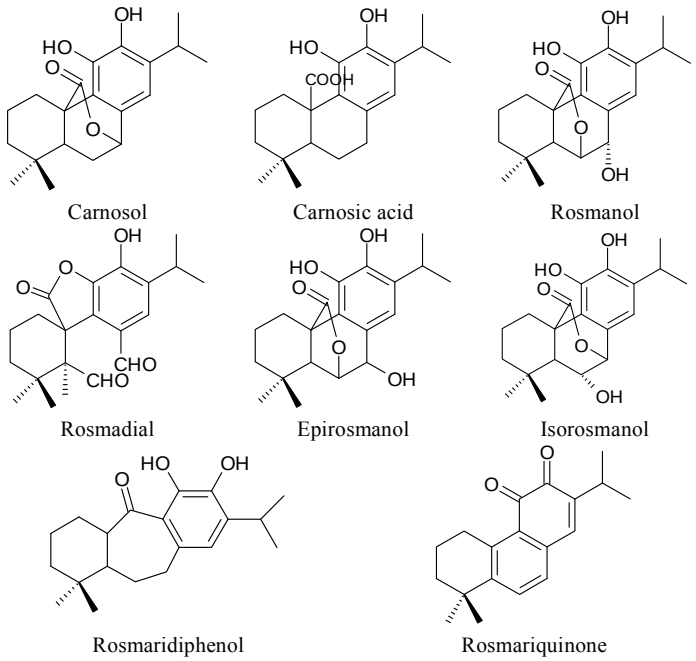

Figure 4. Phenolic diterpenes in Rosemary composition

The caffeic and rosmarinic acids associated with flavonoids (luteolin, hesperidin, naringenin, apigenin) represent other important source of Rosemary antioxidants [13-15] (Figure 5).

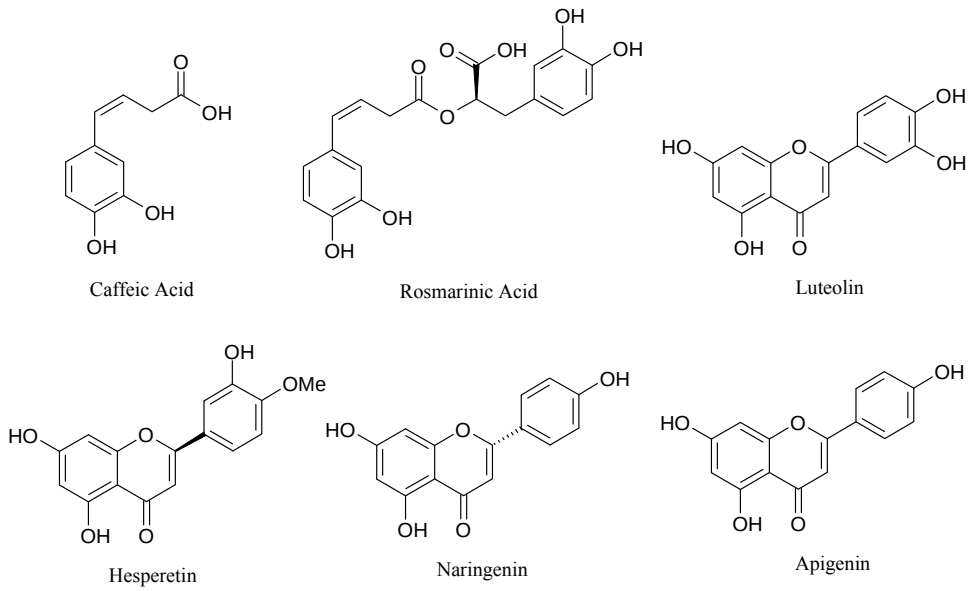

Figure 5. Phenolic acids and flavonoids in Rosemary composition

The reaction of these compounds with peroxy radicals follows the scheme:

$$R\dot{O}_2 + AH\,(\text{antioxidant}) \longrightarrow ROOH + \dot{A}$$

$$R\dot{O}_2 + \dot{A} \longrightarrow ROOA$$

The outstanding antioxidant activity of the Rosemary extract is the basis on which lies its radioprotective capability which results from the morbidity and mortality analysis of the mice fed with Rosemary extract and irradiated with γ radiation of ^{137}Cs at the dose of 10 Gy. Morbidity watched out for the pathological skin modifications (hair fall) and the appearance of toxic signs (diarrhoea) with the mice batches under study. Due to the increase in the Rosemary extract dose given to the mice batches, a delay in the occurrence time of mice morbidity and mortality was recorded as shown in Figure 6.

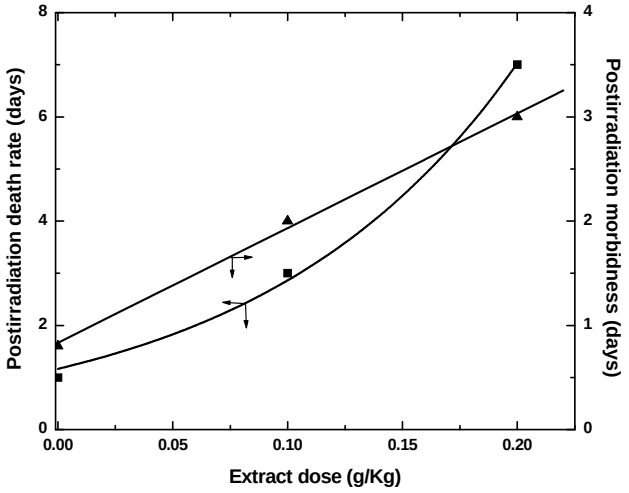

Figure 5. The delay in the mice morbidity and mortality after γ ^{137}Cs (10Gy) irradiation due to the increase of the administrated Rosemary dose

The ionizing radiation induce the lipid peroxidation chain reactions, which damage DNA and the cell. The phenolic antioxidants of the Rosemary extract, taken before irradiation, significantly reduce lipid peroxidation [16] (Figure 7).

INITIATION:	$Lipid + R^{\bullet}$ (or $HO^{\bullet}$) $\rightarrow (Lipid)^{\bullet}$
PROPAGATION:	$(Lipid)^{\bullet} + O_2 \rightarrow Lipid\text{-}OO^{\bullet}$ $Lipid\text{-}OO^{\bullet} + Lipid \rightarrow Lipid\text{-}OOH + (Lipid)^{\bullet}$
TERMINATION:	$(Lipid)^{\bullet} + (Lipid)^{\bullet} \rightarrow Lipid\text{-}Lipid$ $Lipid\text{-}OO^{\bullet} + (Lipid)^{\bullet} \rightarrow Lipid\text{-}OO\text{-}Lipid$
TRAPPING:	$(Lipid)^{\bullet} + Antioxidant \rightarrow Lipid + (Antioxidant)^{\bullet}$

Figure 6. Lipid oxidation

Another hypothesis considers the increased level of glutathione in the blood for Rosemary pre-treated animals; this prevents lipid peroxidation [16-19]. Rosemary extract proved to be an effective radioprotector in animals exposed to gamma irradiation dose of 10 Gy.

4. Conclusions

Rosemary extract shows a remarkable activity in stabilization of organic substrate.

Rosemary extract could offer protection against the effects of ionizing radiation because of its ability to scavenge free radicals.

Phenolic diterpenes, caffeic and rosmarinic acids associated with flavonoids in Rosemary extract suppress lipid peroxidation and stop oxidative DNA damage and so it may be useful as radioprotector agent.

Chemiluminescence has proved its versatility in fast and accurate assessment degradation study.

References

[1] Aruna, K., Sivaramakrishnan, V. M., *Indian J. Experim. Biol*., **28**, 1008, 1990.
[2] Ho, C.T., Wang, M., Wei, G. J., Huang T. C., Huang, M. T., *Biofactors*, **13**, 161, 2000.
[3] Singletary, K., MacDonald, C., Walling, M., *Cancer Lett*., **104**, 43, 1996.
[4] Cheung, S., Tai, J. *Oncol. Rep*., **17**,1525, 2007.
[5] Steiner, M., Priel, I., Giat, J., Levy, J., Sharoni, Y., Danilenko, M., *Nutr. Cancer*, **41**,135, 2001.
[6] Offord, E.A., Mace, K., Avanti, O., Pfeifer, A.M., *Cancer Lett*., **19**, 114, 1997.
[7] Hanif, R., Qiao, L. Shiff, S.J., Rigas, B., *J. Lab. Clin. Med*., **130**, 576, 1997.
[8] Jiang, M.C., Yang-Yen, H.F., Yen, J.J., Lin, J.K., *Nutr. Cancer*, **26**, 111, 1996.
[9] Chuang, S.E., Kuo, M.L., *Carcinogenesis*, **21**, 331, 2000.
[10] Mishima, S., Saito, K., Maruyama, H., Inoue, M., Yamashita, T., Ishida, T., Gu, Y., Biol. *Pharm. Bull*., 27, 1004, 2004.
[11] ***, http://www.buzzle.com/articles/herbs-for-cancer.html
[12] Aruoma, O.J., Halliwell, B., Aerschbach, R., Loliger, J., *Xenobiotica,* **22**, 257, 2002.
[13] Zgorka, G., Glowniak, K., *J. Pharm. Biomed. Anal.,* **26**, 79, 2001.
[14] Justensen, U., Knuthsen, P., *Food Chem*., **73**, 245, 2001.
[15] Zheng, W., Wang. S. Y., *J. Agric. Food Chem*, 49, 5165, 2001.
[16] Sancheti, G., Goyal, P.K., *Afr. J. Trad. CAM* **4**, 165, 2007.
[17] Jindal, A., Soyal, D., Sancheti, G., Goyal, P. K., *J. Envir. Path. Toxicol., Oncol*., **25**, 633, 2006.
[18] Acharya, S. G., Goyal, P. K., *Nuclear Technol. & Rad. Protection* **23**, 72, 2008.
[19] Sancheti, G., Goyal, P. K., *Trees for Life Journal***, 2**, 2, 2007.

Manuscript received: 14.08.2009 / accepted: 26.09.2009