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Chromatographic analyses of *Lavandula angustifolia* and *Rosmarinus officinalis* extracts and their biological effects in mammalian cells and cell-free systems

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Knowledge of biological properties of natural compounds allows to understand their therapeutic value, efficacy and security. We investigated: composition of *Lavandula angustifolia* (LA) and *Rosmarinus officinalis* (RO) extracts, their antioxidant capacity, cytotoxicity and genotoxicity, their DNA-protective potential against DNA damage induced in hamster V79 cells by several genotoxins or in plasmid DNA by Fe^{2+} ions and activity of antioxidant enzymes in cells treated with these extracts. Higher cytotoxicity, observed at higher concentrations of extracts, was accompanied by the increased level of single-strand (ss) DNA breaks as well as formamidopyrimidine DNA glycosylase (Fpg) sensitive sites. LA and RO extracts were able to protect DNA of hamster cells as well as plasmid DNA against ss DNA breaks induced by genotoxins and Fe^{2+}. LA extract mildly increased the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), while RO extract decreased the activity of SOD, but increased the activity of CAT and GPx. Cell-free tests confirmed antioxidant activity of both extracts. The biological properties of LA and RO extracts showed that they could have a positive impact on human health.

Key words: Lavandula angustifolia extract, Rosmarinus officinalis extract, antioxidant activity, DNA protectivity, Comet assay, DNA topology assay

Aromatic plants have been used for many generations in the prevention of diseases or in relieve of their symptoms [1]. It is remarkable that in recent times natural products are often used as starting points for development of drugs. They were the inspiration for approximately one half of drugs used nowadays in the USA and approved by the US FDA (Food and Drug Administration).

Lavandula angustifolia (LA) and *Rosmarinus officinalis* (RO) represent medicinal aromatic plants rich in pharmacologically active substances. Both species are high in essential oils, the major constituents of which are terpenes [2, 3] and also di- and triterpenoids, flavonoids and phenolic acids displaying high antioxidant effects [2-5]. LA was originally found only in the Mediterranean, but now it blooms in many sunny locations around the world and it is also very popular as a decorative garden shrub. RO is thought to be originated in the Mediterranean region as a wild, strewing evergreen perennial shrub. Today, it is grown in nearly all parts of the Mediterranean region and Asia. LA and RO are used for many purposes including medicine, food industry, dyeing, repellents, fragrances and cosmetics [6-8]. The continuing and growing human exposure to natural products has led to an enhancement of the scientific interest about their biological effects. It was found that under certain conditions plant products induce adverse effects (genotoxicity, cytotoxicity) [9]. On the other hand, numerous evidences indicate that diet containing various plant extracts is inversely proportional to the risk of chronic degenerative diseases. This points to antimutagenic and anticarcinogenic effects of plant extracts [10]. Resultant effect is the outcome of interaction between these opposing forces, which can be modified by a large number of exogenous and endogenous factors.

Mammalian cells are continuously subjected to physiological and external influences which can give rise to perilous oxidative damage. The integrity of DNA is vital to cell division, therefore oxidative alterations can disrupt transcription, translation, DNA replication and may result in mutations, cell senescence and death [11]. DNA damage induced by reactive oxygen species (ROS) resulted in DNA base modifications, single- and double-strand breaks and the formation of apurinic/apyrimidinic (AP) lesions [12, 13]. Various chemicals can cause oxidatively generated DNA damage. Hydrogen peroxide (H_2O_2) induces DNA strand breakage by forming hydroxyl radicals (·OH) close to the DNA molecule via the Fenton reaction in the presence of transition metal ions [14, 15]. Organic hydroperoxides, such as *tert*-butyl hydroperoxide (*t*-BHP), may induce DNA damage by promoting the formation of alkali-labile (AL) sites and ss breaks. Iron-dependent processes reflecting Haber-Weiss chemistry play a major role in the formation of free-radical intermediate(s), which could ultimately generate DNA lesions in cells challenged with *t*-BHP [16, 17]. 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) induces in cells both frank DNA breaks and oxidatively generated DNA lesions [18, 19]. Two mechanisms have been proposed to explain quinone cytotoxicity (i) oxidative stress via the redox cycle and (ii) the arylation of intracellular nucleophiles. DMNQ enters into oxidation-reduction reactions (via one-electron-based redox cycling), but has no arylating moiety in its structure [20, 21]. Methyl methanesulfonate (MMS) is a monofunctional direct-acting alkylating agent, active in all of the standard short-term tests aimed at genetic effects. MMS alkylates directly the nitrogen and oxygen atoms of DNA bases and the oxygen moieties of the phosphate backbone. Alkylation of cellular macromolecules by reactive electrophiles contributes to chemical toxicity and oxidative stress of cells which causes frequently depletion of intracellular nucleophilic tripeptide glutathione (GSH) [22]. Lesions induced in DNA by the all above-mentioned chemicals can be revealed by the conventional or modified comet assay. These lesions represent mainly breaks at AP sites or Fpg sensitive lesions [23, 24].

Plant extracts, containing essential oils (EOs) and their components, have been investigated from various aspects but there is still little information about mechanisms of their preventive effects. Suppression of interaction between genes and reactive genotoxins or inhibition of metabolic activation of indirectly acting genotoxins is considered as possible ways of genotoxicity inhibition. In order to evaluate harmful and protective effects of LA and RO extracts we characterized their composition and investigated antioxidant capacity, cytotoxicity and genotoxicity, their DNA-protective potential against DNA damage induced in hamster V79 cells by several genotoxins (H_2O_2, *t*-BHP, DMNQ and MMS) or in plasmid DNA by Fe^{2+} ions and activity of antioxidant enzymes in cells treated with these extracts.

Materials and methods

Chemicals and materials. The extracts of *Lavandula angustifolia* (raw material flowers) and *Rosmarinus officinalis* (raw material leaves) were produced by supercritical fluid extraction with natural carbon dioxide and by hydro-alcoholic extraction, respectively, analysed and certified by Flavex® Naturextrakte GmbH (Germany). GCMS analytical results for Lavender flower extract (type no. 084.001, batch no. 701407, lab no. 10261) were: content of essential oil 68.7%; volatile components: 30.1% linalyl acetate, 25.4% linalool, 6.5% 1,8-cineole, 2% lavandulyl acetate, 0.8% caryophyllene, 0.75% α-terpineol, 0.4% lavandulol, 0.18% ocimene, 0.16% limonene, 0.1% myrcene, 0.05% p-cymene as specified by manufacturer. HPLC analytical results for Rosemary antioxidant extract (type no. 027.004, batch no. 221305, lab no. 13261) were: carnosic acid 14.5%, carnosol 7.7%, ursolic acid 3.1%, 12-methyl-carnosic acid 2.5%, oleanolic acid 1.8%, 7-methyl-rosmanol 0.91%, rosmanol 0.34% as specified by manufacturer. Extracts were kept at 4°C and diluted in appropriate reaction mixtures just before the experiments.

Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (*t*-BHP), 2,3-dimethoxy-1,4-naphthoquinone (DMNQ), methyl methanesulfonate (MMS), 2,2'-azinobis(ethyl-2,3-dihydrobenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), carnosic acid, rosmarinic acid and apigenin were purchased from Sigma-Aldrich Co. Authentic reference substances of α-pinene, β-pinene, β-myrcene, γ-terpinene, terpinolene, β-ocimene, *p*-cymene, 1,8-cineole (eucalyptol), linalool oxide (mixture of isomers), linalool, α-terpineol, terpinen-4-ol, nerol, fenchone, camphor, linalyl acetate and *n*-alkanes standard solution (C_6-C_{30}) as well as a 50/30 μm divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fiber (2 cm length) were also provided by Sigma-Aldrich Co. RANSOD kit was purchased from Randox Laboratories Ltd. (Crumlin, UK) and glutathione reductase, glutathione, NADPH, gallic acid (GA), butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2EDTA \times 2H_2O$), dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ethidium bromide (EtBr) from Sigma-Aldrich Co. DMEM medium and chemicals used for cell cultivation were purchased from Gibco BRL (Paisley, UK), Fpg from Bio-Labs Inc., New England and phosphate-buffered saline (PBS; Mg^{2+}- and Ca^{2+}-free) from OXOID LIMITED, Basingstoke, UK. All other reagents and chemicals used were of analytical grade.

Chromatographic analyses of LA and RO extracts. In order to characterize the volatile profile of both analyzed extracts the headspace solid phase microextraction (HS-SPME) followed by two-dimensional (GC × GC) gas chromatography was applied. The appropriate amounts of the samples (1000 mg of LA extract and 10 mg of RO extract) were transferred to 10 ml screw-top headspace vials and incubated at 50°C in a heating unit of autosampler for 5 min with an agitation of

700 rpm before extraction. Then HS-SPME was performed at 50°C for 30 min using a DVB/CAR/PDMS fiber. After the extraction, the SPME fiber was automatically removed from the sample vial headspace and introduced into the GC × GC injector port for desorption at 250 °C for 5 min. The post-bake out of SPME fibre in a conditioning station of autosampler at 250°C for 7 min was carried out in order to avoid possible sample carryover. The analyses of obtained SPME extracts were performed using the GC × GC system consisting of an Agilent 7890A gas chromatograph, equipped with liquid nitrogen-based quad-jet dual-stage cryogenic modulator and a secondary oven, coupled with Pegasus 4D time-of-flight mass spectrometer (TOFMS; LECO Corp., St. Joseph, MI, USA). The column set was arranged in nonpolar-polar manner as described earlier Kupska et al. [25]. The separation of sample components was performed using the corresponding temperature program. The TOFMS (time-of-flight mass spectrometry) was operated in the electron impact ionization mode at 70 eV with the electron voltage set to -1650 V. The data were collected over a mass range of 33-450 amu at an acquisition rate of 100 spectra/s. The obtained total ion current chromatograms were processed according to data analysis described by Kupska et al. [25]. In the case of RO extract, the characterization of phenolic compounds was done in methanolic extracts (0.3 g of powder in 1 ml of methanol) with the use of Agilent 1200 series HPLC system with DAD detector and ESI interface and mass spectrometer (MS) (Agilent 6130 Quadrupole LC/MS). Chromatographic separation was carried out using Phenomenex Kinetex XB-C18 100A column (150×4.6 mm, 5 µm particle size). The resolution of phytochemicals was carried out using a mobile phase composed of 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B) at a flow rate of 0.8 ml/min; the injection volume of all samples was 2 µl. The elution gradient profile used was 60-100% B in 10 min, followed by 10 min 100% B. MS parameters were as follows: capillary voltage, 3000 V; fragmentor, 120 V; drying gas temperature, 350°C; gas flow (N_2), 12 l/min; nebulizer pressure, 35 psig. The instrument was operated both in positive and negative ion modes, scanning from m/z 100 to 1000. Individual phenolic compounds were identified by comparing their retention times with those for standards or on the basis of available literature data and UV and mass spectra. Flavones were quantitated as apigenin and phenolics diterpens as carnosic acid. The post-column derivatisation was done according to Kusznierewicz et al. [26].

V79 cell line and treatment of cells. Chinese hamster V79 lung fibroblasts were obtained from Prof. A. Abbondandolo (National Institute for Cancer Research, Genova, Italy) and cultured in DMEM, supplemented with 10% FBS and antibiotics (penicillin 200 U/ml, streptomycin and kanamycin 100 µg/ml) in plastic Petri dishes at 37°C in humidified atmosphere of 5% CO_2.

LA extract was disolved in DMSO and diluted to final concentrations of 0.0125-1 µl/ml in complete DMEM. RO extract was dissolved in 70% ethanol and DMEM to final concentrations of 1-100 µg/ml. Cells were cultivated in the presence of LA or RO extracts for 18 h. Control cells were kept in complete DMEM with addition of DMSO or ethanol at concentration which never exceeded 1%.

H_2O_2 was diluted in complete DMEM to a final concentration of 0.6 mmol/l and *t*-BHP in serum-free DMEM to a final concentration of 70 µmol/l. DMNQ was stored as a 50 mmol/l stock solution at -20°C and dissolved before use in serum-free DMEM to a final concentration of 50 µmol/l. It was applied on cells with or without metabolic activation mix S9 [27, 28] after dilution 1:10 in serum-free DMEM containing NADP (4 mmol/l) and glucose-6-phosphate (5 mmol/l) as suggested Maron and Ames [29]. MMS was diluted in complete PBS with Ca^{2+} and Mg^{2+} to a final concentration of 0.4 mmol/l. V79 cells (1×10^6) were treated with the above-mentioned agents for 30 min at 37°C.

Cytotoxicity of LA and RO extracts. Exponentially growing V79 cells (96-well plates in a density of 5×10^3/well) were treated with LA (0.0125-1 µl/ml) or RO (1-100 µg/ml) extracts or without them (control) for 18 h and used for testing of cytotoxicity by the MTT assay. This represents a colorimetric method for measuring the activity of mitochondrial enzymes that reduce MTT, a yellow tetrazole, to purple formazan [30]. Modification used in our experiments described Melusova et al. [31]. Absorbance (A) at a wavelength of 540 nm was measured using an xMark™ Microplate Spectrophotometer (Bio-Rad Laboratories, Inc.) and background absorbance at 690 nm was subtracted. The viability of V79 cells was calculated by the following formula: Viable cells (%) = $A_{\text{treated cells}}/A_{\text{control cells}} \times 100$

Conventional and modified single cell gel electrophoresis (SCGE; Comet assay). Assessment of DNA damage in V79 cells was performed by the use of SCGE according to Singh et al. [32] and followed with minor modifications made by Slamenova et al. [33] and Gabelova et al. [34]. In brief, appropriate number of control or treated V79 cells was trypsinized, centrifuged (1000 rpm, 5 min) and re-suspended in 0.75% low-melting-point agarose in PBS. Approximately 3×10^4 cells was spread on a microscopic slide pre-coated with 1% normal-melting-point agarose in distilled H_2O and covered with cover slips. When the agarose had solidified, the samples were placed in lysis mixture (2.5 mol/l NaCl, 0.1 mol/l Na_2EDTA, 10 mmol/l Tris-HCl, pH=10.0, 1% Triton X-100) for 1 h at 4°C. After lysis, the slides were transferred into an electrophoresis buffer (0.3 mol/l NaOH, 1 mmol/l Na_2EDTA, pH>13.0) for unwinding (40 min at 4°C). A current of 25 V (0.3 A) was then applied for 30 min. The slides were neutralized with 0.4 mol/l Tris-HCl (pH=7.5) and stained with EtBr (5 µg/ml).

For the detection of Fpg sensitive lesions induced by DMNQ the modified comet assay (described by Collins et al. [23]) was used. After lysis, samples were washed twice in endonuclease buffer (40 mmol/l Hepes, 100 mmol/l KCl, 0.5 mmol/l EDTA, pH=8.0) for 10 min and incubated for 30 min with Fpg in a moist box at 37°C. The final dilution of Fpg was 0.2 U/slide. The control slides were incubated with endonuclease buffer containing 0.2 mg/ml BSA. The follow-

ing steps of unwinding, electrophoresis, neutralization and DNA staining were identical in both the conventional and modified technique.

For each sample, at least 100 EtBr-stained nucleoids were evaluated and scored with a Zeiss Imager.Z2 fluorescence microscope using automated computerised image analysis (Metafer 3.6, MetaSystems GmbH, Altlussheim, Germany) for the determination of percentage DNA in the tail.

DNA topology assay. The method of electrophoretically monitored DNA protectivity and antimutagenicity was described in detail by Cipak et al. [35]. Briefly, the reaction mixture (final volume of 10 µl) contained 200 ng of plasmid DNA in buffer and either Fe^{2+} alone, or tested extracts alone, or combinations of tested extracts with Fe^{2+}. Fe^{2+} ions induce DNA breaks via Fenton-like reaction that results in a free radicals formation. The assay responds sensitively to a free-radical generation in the reaction medium. Increase of DNA strand breaks was assayed by measuring the conversion of supercoiled DNA, form I, to a relaxed form II. Analysis of DNA modifications was made by agarose gel electrophoresis (1.5% agarose, 60 min/60 V). The DNA was visualized by staining with EtBr (1 mg/ml) and UV illumination (UV Transilluminator MiniBISPro, DNR Bio Imaging Systems Ltd.).

Antioxidant enzymes activity assays. V79 cells were treated with different concentrations of LA or RO extracts for 18 h. For the determination of SOD (EC 1.15.1.1) and GPx (EC 1.11.1.9) activities, we used 3×10^4 treated and control V79 cells, which were solved 1:1 in 0.1% Triton X-100. For the determination of SOD, we used 1.5×10^4 of V79 cells and the RANSOD kit. The method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The activity is measured by the degree of reaction inhibition. For GPx determination according to the method of Paglia and Valentine [36], we used 3×10^4 V79 cells and cumene hydroperoxide as a substrate.

CAT (EC 1.11.1.6) activity was determined according to Goth [37]. Samples of 5×10^5 V79 cells were incubated with H_2O_2, as a substrate, at 37°C for 60 s. The enzymatic reaction was stopped with ammonium molybdate and the yellow complex of molybdate + H_2O_2 was measured at 405 nm. Specific activity of CAT was expressed as U/mg protein. One unit of CAT activity was defined as the amount of enzyme that decomposes 1 µmol/l of H_2O_2/min. Similarly the activities of SOD and GPx were expressed as U/mg of protein.

The protein concentrations were determined using the Bradford method. Activities of all antioxidant enzymes were calculated as multiple of relative enzymes activity of control (REAC) = 1.

In untreated V79 cells the activity of SOD represents approx. 1274 U/mg of protein, the activity of GPx represents approx. 36 U/mg of protein, and the activity of CAT represents approx. 400 U/mg of protein. These values were changed (more or less) in those cells which were incubated in the presence of LA or RO extracts for 18 h.

Reducing power assay. The reducing capacity of LA and RO extracts were determined according to Yen and Chen [38] and Zhao et al. [39]. The presence of reducing agents (i.e. antioxidants) induces the conversion of the Fe^{3+}/ferricyanide complex onto the ferrous forms. In brief, different concentrations of LA and RO extracts and GA, used as a positive control, were mixed in 1 ml of methanol with 2.5 ml phosphate buffer (0.2 mol/l, pH=6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixtures were incubated at 50°C for 20 min. Trichloracetic acid (2.5 ml, 10%) was added to each mixture and centrifuged at 3000 rpm for 10 min. Finally, the upper layer of supernatants (2.5 ml) were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) and the absorbances were recorded at 700 nm (GENESYS 10 Bio, Spectronic).

Hydroxyl radical (·OH) scavenging activity. For determination of scavenging activity of ·OH these were generated in an L-ascorbic acid/$CuSO_4$ system. The assay is based on quantification of cytochrome c oxidation [40]. In the experiment, ·OH were generated in 1 ml of 0.15 mmol/l sodium phosphate buffer (pH=7.4) containing 100 µmol/l L-ascorbic acid, 100 µmol/l $CuSO_4$, 12 µmol/l cytochrome c and the samples of extracts to be tested. The mixture was incubated at 25°C for 90 min. The change in transmittance caused by the color change of cytochrome c was measured at 550 nm using a spectrophotometer (GENESYS 10 Bio, Spectronic). The inhibition of ·OH generation by 500 µg/ml thiourea was taken as 100%. BHT was used as a positive control. The inhibition ratio was calculated using the following formula:

$$\text{Scavenging of OH radicals (\%)} = [(T - T_2)/(T - T_1)] \times 100$$

T was the transmittance of ·OH generation system and T_1 and T_2 were the transmittances of the control (no ·OH generation) and test systems, respectively.

Fe^{2+}-chelating activity assay. The results obtained by the above-mentioned techniques were verified by the Fe^{2+}-chelating activity assay, as one of the mechanisms of antioxidant action could be the transition metals chelation. Fe^{2+}-chelating assay uses an iron reagent ferrozine which forms complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted resulting in the colour reduction. The chelating activity of extracts and a positive control ($Na_2EDTA\times2H_2O$) toward ferrous ions was studied by the method described by Rajic et al. [41].

Statistical analysis. The results are expressed as means ± standard deviations (SD) from at least three independent experiments carried out in triplicates. Data were analysed by SPSS 15.0 software. One-sample Kolmogorov-Smirnov test was used to test normality of data distribution. Because all data sets were normally distributed the independent samples *t*-test was performed to test for significant differences between groups. Differences between more than two groups were assessed by one way analysis of variance (ANOVA) followed by the Bonferroni test if equal variances were assumed or Tamhane´s test if equal variances were not assumed. Differences with $p<0.05$ are considered to be statistically significant.

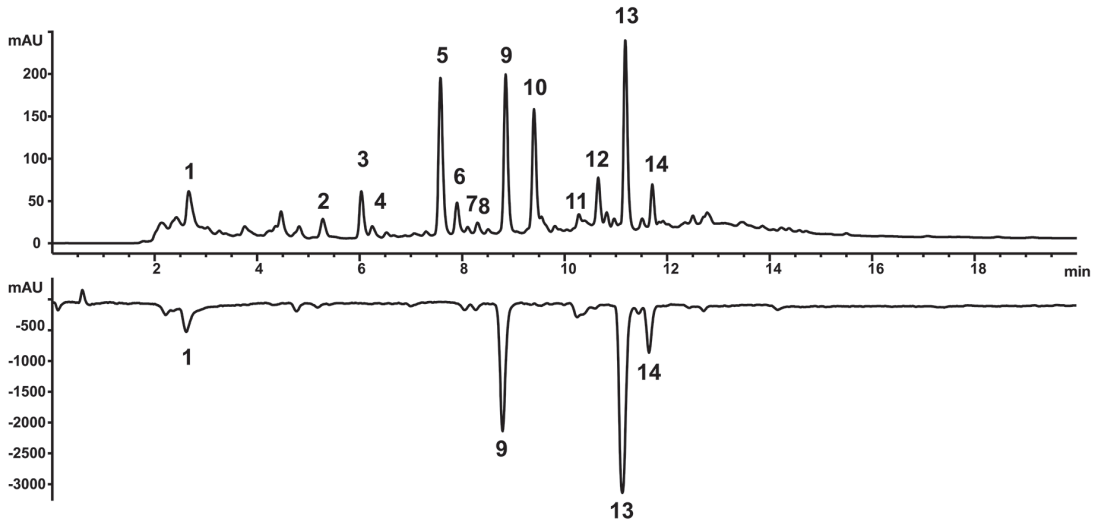

Figure 1. The sample HPLC chromatograms of phenolic compounds (270 nm) obtained for RO extract along with the profiles of antioxidants detected on-line with ABTS (743 nm). Compound names corresponding to each peak is given in Table 1.

Results

Chemical composition of LA and RO extracts. Chromatograms of RO extract recorded before and after derivatization 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) are shown in Figure 1. Table 1 compiles the data for main detected peaks. On the basis of chromatographic and spectrometric data of available standards 3 compounds were identified, further 10 structures were deduced according to peaks' UV and MS spectra and literature data. Among these 13 compounds, there were 1 hydroxycinnamic acid, 2 flavons and 10 phenolic diterpens. The mass spectra collected during analysis reveal fragmentation patterns characterized by a major molecular ion peak (Table 1). The quantitative estimation of phenolic compounds shown in Table 1 was calculated as concentration in dry mass of extracts [mg/g]. Since reference compounds were not available for most of the detected phenolics, flavones were quantitated as apigenin and phenolic diterpens as carnosic acid. In the RO extract studied the carnosic acid and carnosol were the most abundant compounds, although rosmadiol, rosmanol and methyl carnosic acid were also present in a lower proportion. The ability of

Table 1. Composition and content (mg/g d.w.) of phenolic compounds detected in rosemary extract compiled with chromatographic and spectrometric data obtained during HPLC-DAD-MS analyses.

Peak no	Rt [min]	Diagnostic ions		Molecular weight	Identification	Content (mg/g d.w.)
		Positive mode	Negative mode			
1	2.7	383	359	360	Rosmarinic acid (HCA)	7.55 ± 0.70
2	5.3	-	269	270	Apigenin (Flavon)	2.69 ± 0.24
3	6.0	347	345	346	Rosmanol (PDT)	36.22 ± 2.93
4	6.2	347	345	346	Epirosmanol (PDT)	14.13 ± 1.33
5	7.6	285	283	284	Genkwanin (*o*-methylated flavon)	9.34 ± 0.95
6	7.9	361	359	360	Methoxycarnosol (PDT)	27.00 ± 0.96
7	8.1	361	359	360	Epirosmanol methyl ether (PDT)	10.89 ± 0.86
8	8.3	347	345	346	Epiisorosmanol (PDT)	17.24 ± 2.19
9	8.9	331	329	330	Carnosol (PDT)	102.32 ± 9.30
10	9.4	-	343	344	Rosmadiol (PDT)	78.06 ± 2.94
11	10.3	317	315	316	Rosemarydiphenol (PDT)	23.63 ± 1.06
12	10.9	283	289	-	NI	-
13	11.2	355	331	332	Carnosic acid (PDT)	115.91 ± 3.12
14	11.7	-	345	346	Methylcarnosic acid (PDT)	33.27 ± 2.09

HCA, hydroxycinnamic acids; PDT, phenolic diterpenes; NI-not identified.

an antioxidant to quench free radicals by hydrogen donation was exploited for the on-line HPLC-coupled profiling of antioxidants in RO extract. The example of chromatogram obtained during on-line antioxidant profiling with ABTS as derivatization reagent are presented in Figure 1. The post-column detection of the ABTS radical reduction in relation to the content of antioxidants detected is reflected by the negative chromatogram at 734 nm. The obtained results indicate that main contribution to the total antioxidant activity of RO extract has phenolic diterpens (carnosic acid 37%, carnosol 22%, methyl carnosic acid 13%). The share donated by rosmarinic acid (8%) is markedly smaller.

The RO and LA extracts were also analyzed using GC × GC-TOFMS. The chromatographic analyzes revealed complex volatile profiles (Supplementary Table S1) represented mainly by terpene hydrocarbons and their oxygenated derivatives, which constituted 94.3 and 95.5% of the total volatile compounds in LA and RO extracts, respectively. The major class of terpenes/terpenoids identified in RO extract was terpene ketones (44.9%), while LA extract was characterized by the highest relative content of monoterpenols (32.1%). Among 71 compounds identified in LA extract, the most predominant components were linalool (23.3%), camphor (17.0%), linalyl acetate (16.9%) and eucalyptol (10.8%). In the case of RO extract only 43 volatile aroma constituents were detected. Among them, four terpenoids: camphor (41.6%), borneol (19.5%), eucalyptol (7.9%) and bornyl acetate (6.8%) were found to be the most abundant volatile compounds determined in this extract. Both studied plant extracts were also characterized by a low content of other volatile organic compounds, including alcohols, aldehydes, ketones and esters. The main oxygen-containg aliphatic hydrocarbons found in LA and RO extracts were 1-octen-3-yl acetate (2.2%), 3-octanone (1.1%) and hexanal (1.9%), respectively.

Cytotoxic and DNA-damaging effects of LA and RO extracts. The curves in Figure 2 presenting results of the MTT assay following 18 h treatment of V79 fibroblasts with LA (0.0125-1 µl/ml) and RO (1-100 µg/ml) extracts show that IC_{50} values represent the concentrations of 0.6 µl/ml for LA (Figure 2A) and 25 µg/ml for RO (Figure 2B) extracts, respectively. Cytotoxicity of LA and RO extracts at increasing concentrations was in V79 cells accompanied by increased levels of both ss DNA breaks and Fpg sensitive sites measured by the modified comet assay (Figure 2, columns).

DNA-protective effects of LA and RO extracts on V79 cells treated with mutagens. DNA-protectivity of plant extracts was assessed using concentrations at which cell viability was above 80%. Figures 3 and 4 represent DNA-protective effects of 18 h pre-incubation of V79 cells in the presence of LA (Figure 3) or RO (Figure 4) extracts agaist DNA-damaging agents H_2O_2 (panels A), *t*-BHP (panels B), DMNQ (panels C) and MMS (panels D). All four DNA-damaging agents induced a significant increase of DNA damage in V79 cells (Figures 3 and 4, columns 0). Thus, protective activity of LA and RO

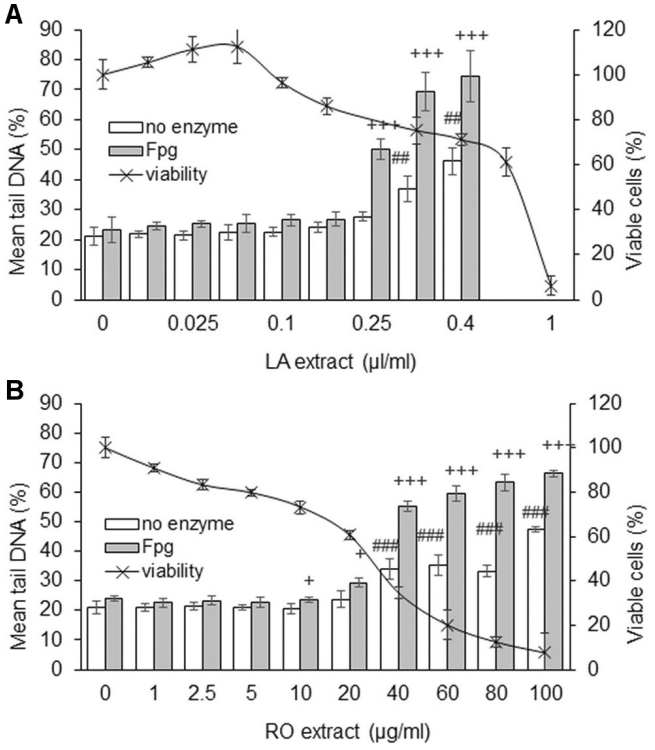

Figure 2. Levels of DNA damage (columns) and viability (curves) in V79 cells treated with LA (A) or RO (B) extracts for 18 h measured by the modified comet assay and MTT test, respectively. Open bars indicate frank ss DNA breaks and AL sites and grey bars symbolize additional Fpg sensitive sites. The data show the means ± SD of 3 independent experiments. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ indicate significant difference compared to the control; $^{+}p<0.05$ and $^{+++}p<0.001$ indicate significant induction of Fpg sensitive sites.

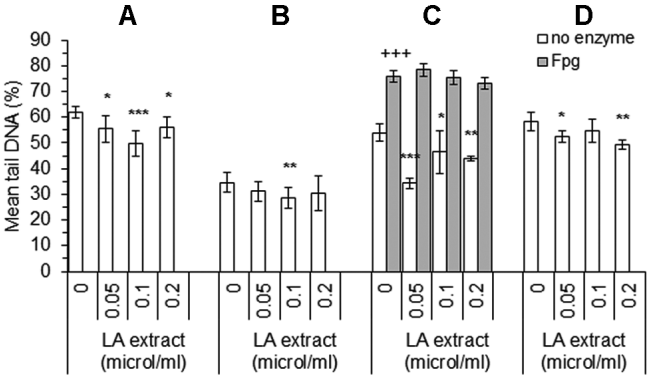

Figure 3. Effects of 18 h pre-treatment of V79 cells with LA extract on DNA damage induced by 0.6 mmol/l H_2O_2 (A), 70 µmol/l *t*-BHP (B), 50 µmol/l DMNQ (C) or 0.4 mmol/l MMS (D) measured by conventional and modified comet assay. Open bars indicate frank ss DNA breaks and AL sites and grey bars symbolize additional Fpg sensitive sites. The data show the means ± SD of 3 independent experiments. $^{+++}p<0.001$ indicate significant induction of Fpg sensitive sites; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ indicate significant difference between V79 cell cultures treated with DNA-damaging agents alone and cultures pre-treated with LA extract and then exposed to H_2O_2, *t*-BHP, DMNQ or MMS.

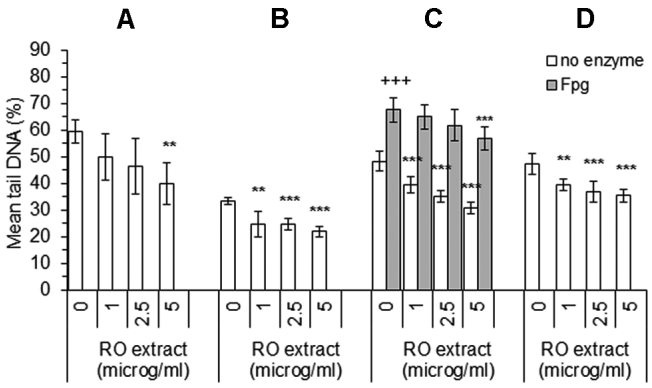

Figure 4. Effects of 18 h pre-treatment of V79 cells with RO extract on DNA damage induced by 0.6 mmol/l H_2O_2 (A), 70 μmol/l *t*-BHP (B), 50 μmol/l DMNQ (C) or 0.4 mmol/l MMS (D) measured by conventional and modified comet assay. Open bars indicate frank ss DNA breaks and AL sites and grey bars symbolize additional Fpg sensitive sites. The data show the means ± SD of 3 independent experiments. $^{+++}p<0.001$ indicate significant induction of Fpg sensitive sites; $^{**}p<0.01$ and $^{***}p<0.001$ indicate significant difference between V79 cell cultures treated with DNA-damaging agents alone and cultures pre-treated with RO extract and then exposed to H_2O_2, *t*-BHP, DMNQ or MMS.

extracts was assessed as the decrease in DNA damage induced by all model agents.

DNA-protective effects of LA and RO extracts observed by DNA topology assay. Figure 5 shows the electrophoretic monitoring of the structure changes induced in plasmid DNA by the treatment with Fe^{2+} and changes in DNA topology after application of plant extracts studied. While Fe^{2+}-treatment of plasmid generated DNA strand breaks (Figure 5A and B, lanes 2), treatment with LA and RO extracts did not induce any DNA breaks (Figure 5A and B, lanes 3-6). As evident from Figure 5, both extracts studied were able to preserve plasmid DNA at all concentrations tested (Figure 5A and B, lanes 7-10).

Effects of LA and RO extracts on antioxidant enzymes activities. V79 cells treated with LA (0.05-0.2 μl/ml) or RO (2.5-20 μg/ml) or without extracts (control) for 18 h were tested for SOD, GPx and CAT activities. The lowest concentration of LA extract (0.05 μl/ml) had no effect on the antioxidant enzymes activities, whereas higher LA concentrations induced significant increase of these activities (Figure 6A). Treatment of cells with different concentrations of RO extract increased GPx and CAT activities significantly, as well. On the other hand, the SOD activity determined in cells treated with RO extract was either not affected or was lower than SOD activity of control V79 cells (Figure 6B).

Reducing power, scavenging and chelating activities of LA and RO extracts. Reducing power, ·OH scavenging and Fe^{2+}-chelating activities of substances tested was measured using three cell-free assays and compared with that of positive controls. Both LA and RO extracts manifested reducing power and ·OH scavenging capacity which were at the same time lower then the activities of both positive controls used. LA and RO extracts were not able to chelate Fe^{2+} (Table 2).

Discussion

Composition and antioxidant activity of LA and RO extracts. The antioxidant activity of plant extracts is generally due to the matter of phenolic abietane diterpenes. Our results obtained analyzing the phenolic diterpens content of the RO extract are consistent with findings of Kontogianni et al. [3] who attributed the antioxidant effect of rosemary extract to the high concentration of carnosic acid or to the synergistic effects of its different constituents. Jordan et al. [5] observed that in RO extracts with different proportions between carnosic acid and carnosol, the two diterpens equally affected *in vitro* antioxidant activity of extracts.

The extracts from RO leaves and LA flowers were also analyzed for volatile profiles. In general, the volatile fraction composition of LA flower extract is consistent with chemical characteristic defined by Shellie et al. [42]. RO extract analyzed can be classified as the camphor chemotype, which is defined by high content of camphor and borneol as well as the absence of fenchene. Both studied plant extracts were also characterized by a low content of volatile organic compounds which is in line with results of previous studies [42, 43].

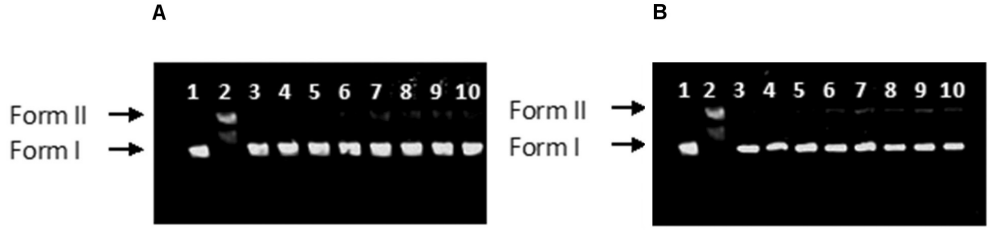

Figure 5. Electrophoretic monitoring of changes induced in pBR322 plasmid by the treatment with Fe^{2+} and changes of DNA topology after application of LA (A) or RO (B) extracts. In electrophoresis pattern A for LA extract represents lane 1: pBR, lane 2: pBR + Fe^{2+}, lane 3: pBR + 100 μl/ml LA, lane 4: pBR + 10 μl/ml LA, lane 5: pBR + 1 μl/ml LA, lane 6: pBR + 0.1 μl/ml LA, lane 7: pBR + 100 μl/ml LA + Fe^{2+}, lane 8: pBR + 10 μl/ml LA + Fe^{2+}, lane 9: pBR + 1 μl/ml LA + Fe^{2+}, lane 10: pBR + 0.1 μl/ml LA + Fe^{2+}. In electrophoresis pattern B for RO extract represents lane 1: pBR, lane 2: pBR + Fe^{2+}, lane 3: pBR + 10 mg/ml RO, lane 4: pBR + 1 mg/ml RO, lane 5: pBR + 0.1 mg/ml RO, lane 6: pBR + 0.01 mg/ml RO, lane 7: pBR + 10 mg/ml RO + Fe^{2+}, lane 8: pBR + 1 mg/ml RO + Fe^{2+}, lane 9: pBR + 0.1 mg/ml RO + Fe^{2+}, lane 10: pBR + 0.01 mg/ml RO + Fe^{2+}.

The total antioxidant activity of a compound has been attributed to various mechanisms, namely (i) inhibition of reactive oxygen/nitrogen species (ROS/RNS) and scavenging capacity; (ii) reducing power; (iii) chelating capacity; (iv) action on antioxidant enzymes; (v) inhibition of oxidative enzymes. A "battery" of assays carried out for evaluating different aspects of the antioxidants was very recommended [44, 45]. To be able to characterize antioxidant activity of LA and RO extracts, we performed several test procedures. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity [46]. Compounds which exhibit reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes [47]. Hydroxyl radicals are potent reactive oxygen species which cause damage to cells in the biological system, therefore it is vital to evaluate whether a tested substance has the capability to defuse these radicals [45]. Iron and other transition metals are able to promote oxidation by acting as catalysts of free radical reactions. Single electrons are transferred by these redox-active metals during changes in oxidation states. Chelation of metals by certain compounds decreases their prooxidant effect by reducing their redox potentials and stabilizing the oxidized form of the metal [48]. Using these cell-free assays we have found out that both LA and RO extracts manifested reducing power and ·OH scavenging capacity but did not display any Fe^{2+}-chelating activity. It is accepted that the antioxidant activity of flavonoids is caused by a combination of iron chelation and free radical scavenging activities. However, van Acker et al. [49] found that this phenomenon does not apply in general, as the majority of flavonoids, which they tested, did not manifest any iron chelation. This observation is in good agreement with findings of Carloni et al. [50] who have described two types of green tea which contained the highest amount of phenols and flavonoids, had the highest antioxidant activity, but at the same time had the lowest chelating activity. The lack of LA and RO extracts chelating activity probably relates to the

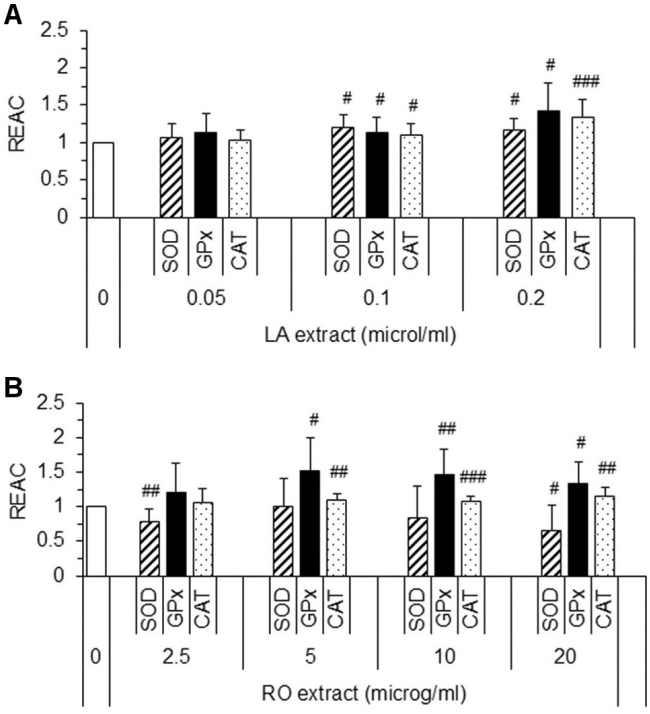

Figure 6. The activity of enzymes SOD (striped bars), GPx (black bars) and CAT (dotted bars) in V79 cells treated with LA (A) or RO (B) extracts for 18 h. REAC was expressed as multiple of relative enzymes activity of control = 1 (1 for SOD=0.764 U/mg, 1 for GPx=0.016 U/mg, 1 for CAT=235.557 U/mg of proteins) ± SD of 3 independent experiments. $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ indicate significant differences compared to the control.

Table 2. Reducing power, hydroxyl radicals (·OH) scavenging and Fe^{2+}-chelating activity of *Lavandula angustifolia* (LA) and *Rosmarinus officinalis* (RO) extracts.

Extracts concentrations	Reducing power (absorbance)	·OH scavenging (%)	Fe^{2+}-chelating (%)
0.01 µl/ml LA	0.089 ± 0.042	13.58 ± 4.21	0.00 ± 0.00
0.1 µl/ml LA	0.094 ± 0.012	15.64 ± 2.75	0.39 ± 0.54
1 µl/ml LA	0.109 ± 0.030	17.18 ± 6.54	1.90 ± 1.68
10 µl/ml LA	0.139 ± 0.027	ND	0.00 ± 0.00
100 µl/ml LA	0.281 ± 0.046	ND	ND
0.001 mg/ml RO	0.063 ± 0.004	15.87 ± 6.24	0.00 ± 0.00
0.01 mg/ml RO	0.071 ± 0.008	13.07 ± 8.72	0.64 ± 0.90
0.1 mg/ml RO	0.102 ± 0.012	17.13 ± 3.63	1.20 ± 1.16
1 mg/ml RO	0.205 ± 0.035	28.50 ± 1.24	0.52 ± 0.76
10 mg/ml RO	0.820 ± 0.125	51.89 ± 3.63	ND
1.25 mg/ml GA	2.339 ± 0.035	–	–
0.25 mg/ml BHT	–	51.13 ± 9.70	–
1 mM $Na_2EDTA \times 2H_2O$	–	–	97.00 ± 0.82

Data represent the means ± SD of 3 independent experiments.
GA, gallic acid; BHT, butylated hydroxytoluene; $Na_2EDTA \times 2H_2O$ are shown as positive controls.
ND – undetectable, sediment was formed in a reaction mixture.

fact that they come from plants containing phenolic acids, flavonoids and triterpenes.

Cytotoxic and genotoxic effects of LA and RO extracts. It is assumed that the cytotoxicity of LA and RO extracts is due to their lipophylic components which are able to pass through the cell walls and in higher concentration disrupt the membranes. Prashar et al. [51] found out that the cytotoxicity of LA oil in human endothelial skin cells and fibroblasts was caused mainly by its major component, the linalyl acetate. Cytotoxicity of RO oil is attributed to carnosic acid and triterpenoids as are betulinic and ursolic acids [3]. Yesil-Celiktas et al. [52] investigated the cytotoxicity of various RO extracts prepared from plants growing in different locations of Turkey and extracted by diverse types of extraction. The extracts exhibited various cytotoxicity against several cell lines. Comparatively low IC_{50} values (12.50-47.55 μg/ml) were found in K562 cells similarly as we determined in V79 cells or Miladi et al. [53] in human cell line A549. Diverse cytotoxicity of RO extracts could be related with the existence of various chemotypes. These are characterized by differences in the quantity of essential oils major components caused by genetic, geographical or environmental factors.

We observed that at concentrations ≥0.25 μl/ml LA and ≥10 μg/ml RO the cytotoxicity of both extracts was in V79 cells accompanied by the increased level of ss DNA breaks and mainly Fpg sensitive sites. Di Sotto et al. [54] examined the genotoxicity of LA oil and its major components in peripheral human lymphocytes using the micronucleus test. Linalyl acetate manifests the highest genotoxic activity. It increased the frequency of micronuclei in a concentration-dependent manner (0.5-100 μg/ml). LA oil was able to induce micronuclei only at the highest concentration and linalool was devoid of genotoxicity completely. These results mean that not only cytotoxic, but also mutagenic activity of LA oil is related to the presence of linalyl acetate. Maistro et al. [6] investigated genotoxicity of RO essential oil using three tests (SCGE, micronucleus and chromosome aberration tests) in liver cells and peripheral blood cells collected from Swiss mice and bone marrow cells isolated from Wistar rats. The authors concluded that this essential oil provokes genotoxic effects in experimental animals when administered orally (once 300, 1000 or 2000 mg/kg).

Antigenotoxic and anticarcinogenic effects of LA and RO extracts. It is known that short-term genotoxicity tests can be also used to identify antigenotoxic agents. We investigated DNA-protective effects of LA and RO extracts by SCGE in hamster lung V79 fibroblasts, which are considered as a suitable model for the assessment of natural compounds protective effects [55]. Pre-treatment of cells with LA and RO extracts for 18 h was able to protect DNA of V79 cells against ss DNA breaks induced by H_2O_2, *t*-BHP and MMS. The protection of cells towards DMNQ-induced DNA lesions is not clear, because the extracts significantly reduced the level of DMNQ-induced ss breaks, but the level of Fpg sensitive sites was reduced only partially (in cells pre-treated with the highest RO extract concentration). We can not explain the reasons of this phenomenon at present. It is possible that oxidatively induced Fpg sensitive sites could be reduced only by stronger antioxidant agents than are LA and RO extracts. It also can not be excluded that the presence of 1,8-cineol, which is known as „an inducer" of Fpg sensitive sites [56], slows the protective ability of the extracts. Evandri et al. [57] did not find any mutagenic effects of LA oil in various types of bacterial strains with or without an extrinsic metabolic activation system. Conversely, LA oil reduced occurrence of mutants in the TA98 strain after exposition by direct mutagens 2-nitrofluorene or 1-nitropyrene. Mitic-Culafic et al. [58] investigated protective effects of LA components against *t*-BHP-induced DNA lesions in human HepG2 and B lymphoid NC-NC cells, and against *t*-BHP-induced reverse mutations in bacteria *Escherichia coli* (WP2). Only linalool, characterized by high radical scavenging activity, reduced significantly all types of genotoxic changes. Repair of *t*-BHP-induced DNA damage in HepG2 cells was not affected by the monoterpens. DNA-protective effects of RO extract were described by various authors. Already in 2002 it was found that long-term or short-term pre-treatment of hamster V79 or human colon cancer Caco-2 cells with extracts of RO manifested protective effects against DNA damage induced by H_2O_2 and visible light-excited methylene blue [59]. The potential DNA-protective effects of RO oil (dissolved in drinking water of rats) were demonstrated in hepatocytes influenced with H_2O_2, visible light-excited methylene blue and DMNQ [18] and in testicular cells treated with H_2O_2 and DMNQ [60]. Melusova et al. [31] studied DNA-protective and pro-apoptotic effect of carvacrol and RO essential oil as well as repair ability of extracts acquired from hepatocytes of rats fed with carvacrol or RO oil. Both volatiles manifested cytotoxic, DNA-protective and pro-apoptotic activity but had no effect on DNA repair in DMNQ-treated HepG2 cells. Zegura et al. [61] found out that water and oil soluble RO extracts efficiently protected human HepG2 cells not only against oxidative stress induced by *t*-BHP but also against indirect-acting mutagens. The authors suggested that the mechanisms of protection could be connected with the modulation of cellular antioxidant responses and detoxication of xenobiotics. Perez-Sanchez et al. [62] discovered that extracts of RO and citrus bioflavonoids reduced harmful UV-effects in human keratinocytes. The combination of flavonoids contained in citrus and polyphenols and diterpenes contained in RO reduces in HaCaT cells intracellular UVB-induced ROS, protects DNA against damage and decreases number of chromosomal aberrations in X-irradiated human lymphocytes. The authors have proposed to use this ingredient for photoprotection. The anticancer properties manifest mainly RO, but the evidence of such activity for LA is rare. Ngo et al. [4] suggested that anticancer properties of RO arise through the molecular changes in the multiple-stage process of cancer development. The authors reached this conclusion on the basis of numerous scientific evidences which appeared in the literature in the period 1996-2010. Anticancer potential of RO extract is ascribed mainly to carnosol and carnosic, ursolic

and rosmarinic acids. Kontogianni et al. [3] presupposed that combination of cytotoxic effect of RO extract (the influence of carnosic acid and triterpenoids betulinic and ursolic acids) and its antioxidant activity (effect of carnosol, rosmarinic and carnosic acids) provides both the direct destruction of cancer cells as well as the protection of healthy cells during cancer treatment.

DNA topology assay. DNA topology assay is based on the fact that topological changes of DNA molecules are able to change electrophoretic mobility of DNA topoisomers, therefore an incidence of DNA breakage changes the topology of plasmid. The assay enables on one side electrophoretic monitoring of DNA breakage induced e.g. by Fe^{2+} ions and on the other side monitoring their reduction after application of suitable DNA-protective compounds as are essential oil borneol [63] or bioactive antraquinone emodin [64]. Our results showed that LA and RO extracts did not change the mobility of the supercoiled plasmid DNA, however, they caused at all concentrations tested a significant reduction of DNA breaks induced by Fe^{2+}. This confirms that LA and RO extracts are able to protect plasmid DNA against damaging effects of Fe^{2+}.

***In vitro* and *in vivo* effects of LA and RO extracts on antioxidant status of cells and tissues.** Cells contain a large number of antioxidant enzymes which are able to protect them against the damage caused by ROS and regulate their redox-sensitive signaling pathways. Three of the primary antioxidant enzymes contained in mammalian cells are SOD, CAT and GPx. The SODs convert superoxide radicals into H_2O_2 and molecular oxygen, whereas the CAT and GPx amend H_2O_2 into oxygen and water. In this way two toxic species, superoxide radical and H_2O_2, are converted to the harmless water products [65]. We have found that in V79 cells, which were incubated in the presence of LA extract (concentration >0.1 µl/ml) was increased mildly the activity of all enzymes tested. LA oil is able to stimulate antioxidant enzymes also in *in vivo* conditions. Wang et al. [66] investigated the reasons of neuroprotective effects of LA oil against ischemia/reperfusion injury in mice. Adding of LA oil to the diet had significantly decreased infarct size, the levels of lipid peroxidation, carbonyl and ROS and attenuated neuronal damage. On the other hand, activities of SOD, CAT, GPx and GSH/GSSG ratio were up-regulated. To similar conclusions came Hancianu et al. [67] investigating the antioxidant and anti-apoptotic activities of LA oils in male Wistar rats subjected to scopolamine, causing dementia. In demented rats LA oil increased activities of SOD, GPx, CAT and content of reduced GSH and reduces a level of lipid peroxidation.

Effects of RO oil seem to be less clear. Incubation of hamster cells with extract of RO (2.5-20 µg/ml) decreased mildly the level of SOD, but significantly increased enzymes CAT and mainly GPx. Posadas et al. [68] investigated antioxidant status of older Wistar rats fed for 12 weeks with a standard kibble containing different concentrations of RO extract (0, 0.02 and 0.2%). Tissue samples collected from heart and brain showed that supplementing the diet of aged rats with RO extract resulted in a decrease of antioxidant enzyme activity, lipid peroxidation and ROS levels. This was significant mainly for CAT activity in heart and brain, nitric oxid synthase in heart and lipid peroxidation and ROS levels in different brain tissues. The authors suggested that although RO supplementation improved the status of oxidative stress of old rats, the antioxidant status of their organs was not improved. Slamenova et al. [60] showed that consumption of RO oil, which increased resistance of rat testicular cells against DNA-damaging effects of H_2O_2 and DMNQ, did not change the activity of antioxidant enzymes GPx and SOD.

The results discussed in previous chapters, which arised on the basis of the extensive experimental work, indicate that LA and RO extracts have numerous beneficial effects and could be suitable and promising candidates for the applications in the human healthcare.

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Supplementary Table S1. Volatile components identified in lavender and rosemary extracts by GC×GC-TOFMS.

Peak No.	Compound name	$LRI_{cal.}$[a]	$LRI_{lit.}$[b]	^1t_r [s][c]	^2t_r [s][d]	Similarity[e]	Reverse[f]	Relative content [%][g]	
								Lavender extract	Rosemary extract
	TERPENES & TERPENOIDS								
	Monoterpenes								
1	Tricyclene	924	921	870	2.07	955	955	0.139	ND
2	α-Pinene[h]	934	934	894	2.10	933	933	0.522	0.463
3	Camphene	947	945	924	2.13	943	945	1.060	0.665
4	Verbenene	950	949	930	2.16	855	866	ND	0.513
5	Sabinene	968	967	972	2.15	942	942	0.111	ND
6	β-Pinene[h]	974	974	984	2.16	885	916	0.575	0.075
7	β-Myrcene[h]	982	982	1002	2.17	952	952	0.289	ND
8	δ-3-Carene	1008	1007	1062	2.14	936	936	0.172	ND
9	γ-Terpinene[h]	1011	1031	1068	2.16	859	873	ND	0.101
10	*p*-Cymene[h]	1013	1012	1074	2.32	936	936	0.594	4.492
11	β-*cis*-Ocimene[h]	1039	1038	1134	2.22	967	967	0.166	ND
12	Dehydro-*p*-cymene	1076	1074	1218	2.56	914	914	ND	0.309
13	Terpinolene[h]	1082	1081	1230	2.21	919	920	0.032	ND
							Total	**3.661**	**6.618**
	Terpene oxides								
14	Eucalyptol[h]	1024	1023	1098	2.35	920	922	10.823	7.894
15	*cis*-Linalool oxide[h]	1063	1065	1188	2.70	889	889	4.115	ND
16	*trans*-Linalool oxide[h]	1076	1076	1218	2.73	955	955	3.575	ND
17	2-Hydroxy-1,8-cineole	1209	1212	1506	3.71	897	897	0.029	ND
18	Caryophyllene oxide	1583	1584	2196	2.38	916	916	0.251	ND
							Total	**18.793**	**7.894**
	Monoterpenols								
19	*trans*-Sabinene hydrate	1061	1061	1182	2.72	875	875	4.182	ND
20	Linalool[h]	1089	1089	1248	3.12	915	915	23.286	ND
21	(R)-Lavandulol	1156	1156	1392	3.06	829	831	0.321	ND
22	Borneol	1158	1161	1398	3.40	896	908	3.628	19.486
23	*p*-Cymen-8-ol	1167	1167	1416	4.06	923	923	0.053	ND
24	Terpinen-4-ol[h]	1169	1170	1422	2.76	927	911	0.241	0.362
25	α-Terpineol[h]	1181	1187	1446	3.06	882	883	0.390	2.284
26	Dihydrocitronellol	1206	1196	1500	2.05	890	894	0.021	ND
27	Nerol[h]	1214	1213	1518	3.27	860	860	0.003	ND
							Total	**32.125**	**22.132**
	Terpene ketones								
28	Fenchone[h]	1074	1075	1212	2.50	895	900	0.003	ND
29	Nopinone	1119	1117	1314	2.98	955	955	0.127	ND
30	Camphor[h]	1131	1132	1338	2.95	919	919	16.976	41.549
31	Isopinocamphone	1144	1147	1368	2.65	855	856	ND	0.717

32	Pinocarvone	1147	1153	1374	2.79	773	849	0.007	0.236
33	Verbenone	1189	1190	1464	3.20	919	919	0.041	2.384
34	Carvone	1223	1223	1536	3.10	950	950	0.040	ND
							Total	**17.195**	**44.887**
	Terpene aldehydes								
35	Myrtenal	1178	1176	1440	2.90	884	912	0.053	0.083
							Total	**0.053**	**0.083**
	Terpene esters								
36	Lavender lactone	1005	997	1056	4.68	952	952	0.502	ND
37	Isobornyl formate	1220	1222	1530	2.58	920	922	0.478	1.141
38	Linalyl acetate[h]	1249	1247	1590	2.52	917	917	16.873	ND
39	Dihydrocarveol acetate	1260	1282	1614	2.42	786	811	0.012	ND
40	Lavandulyl acetate	1271	1272	1638	2.46	909	909	2.738	ND
41	Bornyl acetate	1277	1276	1650	2.42	947	947	0.143	6.788
42	4-Terpinenyl acetate	1289	1289	1674	2.42	874	875	0.003	ND
43	Neryl acetate	1342	1343	1782	2.50	891	895	0.057	ND
44	Geranyl acetate	1361	1362	1818	2.51	895	895	0.085	ND
45	Linalyl isovalerate	1493	1484	2064	2.22	894	911	0.036	ND
							Total	**20.924**	**7.929**
	Sesquiterpenes								
46	α-Copaene	1382	1380	1860	2.15	938	941	ND	0.933
47	β-Bourbonene	1391	1391	1878	2.19	915	915	0.037	ND
48	Isocaryophyllene	1413	1407	1920	2.20	820	827	0.008	0.162
49	α-*cis*-Bergamotene	1417	1415	1926	2.19	929	943	0.045	ND
50	α-Santalene	1423	1424	1938	2.25	929	937	0.520	ND
51	β-Caryophyllene	1427	1428	1944	2.29	940	943	0.436	0.845
52	α-*trans*-Bergamotene	1437	1437	1962	2.19	937	948	0.145	ND
53	Aromadendrene	1450	1452	1986	2.22	905	909	ND	0.372
54	(E)-β-Farnesene	1450	1448	1986	2.24	869	878	0.172	ND
55	Humulene	1460	1458	2004	2.29	865	885	0.022	0.268
56	Alloaromadendrene	1470	1474	2022	2.23	903	907	ND	0.138
57	γ-Muurolene	1480	1481	2040	2.23	890	899	0.005	1.007
58	α-Selinene	1493	1492	2064	2.25	859	867	ND	0.534
59	α-Muurolene	1500	1498	2076	2.20	809	816	ND	0.431
60	Germacrene A	1504	1503	2082	2.25	864	867	0.042	ND
61	γ-Cadinene	1517	1515	2100	2.24	912	926	0.062	0.566
62	Calamenene	1521	1521	2106	2.29	919	932	0.003	0.360
63	δ-Cadinene	1525	1523	2112	2.18	903	905	ND	0.278
64	α-Cadinene	1542	1539	2136	2.19	808	867	ND	0.046
65	α-Calacorene	1542	1539	2136	2.35	893	956	ND	0.046
							Total	**1.495**	**5.984**
	Sesquiterpenols								
66	Cubenol	1616	1617	2238	2.32	762	816	0.001	ND
67	α-Cadinol	1637	1643	2262	2.40	863	902	0.003	ND
							Total	**0.004**	**—**

	ALCOHOLS								
68	1-Penten-3-ol	666	662	426	2.72	894	894	ND	0.091
69	1-Octen-3-ol	966	967	966	2.99	955	955	0.254	ND
70	3-Octanol	982	985	1002	2.65	945	948	0.053	ND
							Total	**0.307**	**0.091**
	ALDEHYDES								
71	Propanal	<600	480	306	2.00	955	955	ND	0.493
72	Pentanal	672	674	432	2.21	899	921	0.014	0.242
73	(E)-2-Pentenal	729	723	504	2.58	886	886	ND	0.135
74	3-Methyl-2-butenal	757	753	546	2.80	923	927	0.040	ND
75	Hexanal	776	778	576	2.31	949	951	0.016	1.893
76	2-Hexenal	829	832	672	2.66	922	922	ND	0.174
77	Heptanal	879	880	774	2.38	918	934	ND	0.088
							Total	**0.070**	**3.024**
	KETONES								
78	2-Butanone	<600	575	348	2.11	955	955	ND	0.504
79	2-Pentanone	666	666	426	2.21	984	988	ND	0.136
80	2-Heptanone	871	872	756	2.40	899	899	ND	0.102
81	3-Octanone	966	965	966	2.40	909	909	1.056	ND
							Total	**1.056**	**0.741**
	ESTERS								
82	Methyl acetate	<600	519	318	2.02	977	979	ND	0.127
83	Methyl propionate	616	618	378	2.10	945	960	ND	0.244
84	Methyl butyrate	706	705	468	2.19	948	949	ND	0.246
85	Butyl acetate	800	798	612	2.24	952	955	0.025	ND
86	Hexyl formate	911	911	840	2.39	961	961	0.035	ND
87	Hexyl acetate	995	995	1032	2.34	953	953	0.701	ND
88	1-Octen-3-yl acetate	1095	1094	1260	2.51	937	940	2.205	ND
89	3-Octyl acetate	1111	1110	1296	2.23	815	839	0.142	ND
90	Hexyl isobutyrate	1133	1135	1344	2.25	936	942	0.320	ND
91	Hexyl butyrate	1175	1175	1434	2.29	938	938	0.588	ND
92	Octyl acetate	1194	1195	1476	2.32	953	953	0.029	ND
93	Hexyl-3-methylbutyrate	1226	1227	1542	2.22	909	913	0.188	ND
94	Hexyl tiglate	1312	1310	1722	2.38	890	948	0.082	ND
							Total	**4.316**	**0.617**

[a] $LRI_{cal.}$ – linear retention indices experimentally determined based on the analysis of C_6-C_{30} *n*-alkanes mixture and using Van den Dool and Kratz's equation (1963); [b] $LRI_{lit.}$ – literature values of linear retention indices obtained under chromatographic conditions similar to those used in this study (column with non-polar stationary phase, 100% polydimethylsiloxane) (NIST Standard Reference Database Number 69); [c] ^1t_r – 1st dimension retention time; [d] ^2t_r – 2nd dimension retention time; [e] forward similarity of mass spectra (value out of 1000); [f] reverse similarity of mass spectra (value out of 1000); [g] relative content calculated based on the areas of chromatographic peaks of detected compounds; [h] compounds identity confirmed by the analysis of authentic standards.