STUDIES ON ANTIOXIDANT PROPERTIES BEFORE AND AFTER UV- AND γ-IRRADIATION OF BULGARIAN LAVENDER ESSENTIAL OIL ISOLATED FROM *LAVANDULA ANGOSTIFOLIA* MILL.

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ABSTRACT

At present there is not enough data about different types of radiation on Bulgarian essential oils isolated from medicinal plants. Here we report for the first time our studies on the effects of UV-radiation (290 nm to 320 nm) and γ-radiation (doses of 5 Gy, 10 Gy, 20 Gy and 30 Gy) on Bulgarian lavender essential oil isolated from Lavandulla angustifolia Mill. A spectrophotometry method was used for evaluation of the changes in the reducing power of the Bulgarian lavender oil before and after radiation. The electron donation potential of non-irradiated oil was found to be lower (0.268 ± 0.0244) than those of both, UV-irradiated (0.336 ± 0.0121) and γ-irradiated, oil samples (0.427 ± 0.0251 at 5 Gy radiation, 0.341 ± 0.0371 at 10 Gy; 0.328± 0.0173 at 20 Gy). By direct electron paramagnetic resonance (EPR) spectroscopy, single almost symmetrical EPR signals with different values of the g-factor were registered in non-irradiated (g = 2.0047 ± 0.0002), UV-irradiated (g = 2.01050 ± 0.00005) and γ-irradiated oil samples (g = 2.0017 ± 0.0002), respectively. EPR spectroscopy was used for assessment of the radical scavenging capacity of the non-irradiated and UV- and γ-irradiated samples of the Bulgarian lavender oil towards the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Excellent DPPH radical scavenging capacities were found for UV- and 30 Gy-irradiated lavender oil samples. Based on these preliminary results, we consider that, after proper UV- or γ-radiation treatment, Bulgarian lavender oil might find application as a good radioprotector and antioxidant in cosmetics and medicine.

Biotechnol. & Biotechnol. Eq. 2013, **27**(3), 3861-3865

Keywords: *Lavandulla angustifolia* Mill., reducing power, DPPH scavenging capacity, EPR spectroscopy, antioxidants, UV-radiation, γ-radiation

Introduction

The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis. Reactive oxygen/nitrogen species (ROS/RNS) play a fundamental role in maintaining the metabolic homeostasis in the human body. The imbalance of production and generation of free radicals (11) or ROS caused by external agents or effects of radiation-therapy stress leads to oxidative stress. Effects of radiation and formation of ROS/RNS might be overcome by using external supplementation of antioxidants of plant (floral) origin (3, 15, 17, 25).

Essential oils, especially lavender oil and their components, exhibit muscle relaxant, antibacterial and antifungal activities, protect against cancer, dementia, depression, stress, sleep disorders, headaches and have demonstrated good antioxidant activity. Antioxidants are compounds that are able to delay, retard or prevent oxidation processes. They can interfere with oxidation by reacting with free radicals, chelating metals and by acting as oxygen scavengers and transferring hydrogen atoms to the free radical structures (4, 13, 19, 20). Recent reports demonstrated the usefulness of flower extracts and plant products as good antioxidants, and as radioprotective agents (especially after UV-/γ-irradiation) that effectively mitigate radiation-induced oxidative stress (2, 6).

The lavenders (Lavandula) are a genus of 39 species of flowering plants in the mint family, *Lamiaceae*, and are extensively used with herbs and for aromatherapy. Bulgarian lavender, class *Lavandula angustifolia* Mill., grows in the Kazanlak region, in black soils, bearing small purple flowers. Bulgarian lavender essential oil is a mixture of over 200 components belonging to the terpene and non-terpene hydrocarbons, alcohols (linalool), aldehydes, ketones, ethers, acids, lactones, phenols, and esters. Linalyl acetate (low acute toxicity, at concentrations from 0.5 μg/mL to 100 μg/mL) is the ingredient determining the quality of lavender oil. Content of linalyl acetate from 25 % to 31.1 % is typical for the quality of Bulgarian lavender oil and meets the standard for French oil. Bulgaria is known for producing high-quality essential lavender oil distilled from fresh clusters of seeds from

populations belonging to the Bulgarian varieties of *Lavandula angustifolia* Mill. (4, 9, 10, 24). One of the new applications of lavender oil is its use for inhalations and in alleviating anxiety and related sleep disturbances. A number of studies performed with *in vitro* and *in vivo* models reported that lavender oil has protective effects against different types of damages (1, 7, 12, 21).

Gamma-irradiation is internationally recognized as an effective method for maintaining the quality of food, spices and oils for a long time. Directive 1999/3/EC established a Community list of food and food ingredients that may be treated with ionizing radiation and the maximum overall average absorbed dose may be 10 kGy for aromatic herbs, spices and vegetable seasonings (8). Essential oils extracted from γ-irradiated fruits have been found to be more effective as antioxidants. Statistical analyses of the effects of irradiation on the content of volatile oils in spices showed that there were significant differences between irradiated and non-irradiated samples (17, 18, 23, 26).

Based on the following important facts that: 1) application of UV- and γ-radiation on natural essential oils causes formation of paramagnetic species; 2) electron paramagnetic resonance (EPR) spectroscopy is considered to be a unique technique for detection and characterization of free radical structures, antioxidant activity and radical scavenging capacity of different natural and synthetic extracts and compounds (16, 18, 22 and 3) insufficient information is available on the effects of UV- and γ-irradiation of Bulgarian essential oils isolated from medicinal plants; the aim of this study was to investigate and compare the changes in the free radical scavenging capacity, antioxidant and radiomodulatory properties of non-irradiated samples and UV- and γ-irradiated Bulgarian (*Lavandula angostifolia* Mill.) lavender essential oil, using a standard *in vitro* spectrophotometrical method and the EPR technique.

Materials and Methods

Chemicals

Lavandula angustifolia Mill., Lamiaceae, oil (density of 92 g/L, and 100 % purity) obtained by steam distillation was provided from the Institute for Roses and Aromatic Plants, Kazanlak, Bulgaria (24). 1,1-dipheniyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemicals, USA. Deionized and distilled water was used for all experiments. Other chemicals used were analytical or HPLC grade.

UV- and γ-irradiation

Lavender oil samples were irradiated using a UV–VIS Transilluminator-4000 (Stratagene, USA). Samples were radiated with UV-light in the wavelength range from 290 nm to 320 nm for 2 h (relative humidity of 40 %). Lavender oil samples were irradiated with γ-rays from a ^{60}Co source at doses of 5 Cy, 10 Gy, 20 Gy, and 30 Gy in a γ-chamber (Gamma cell 5000, Board of Irradiation and Isotope Technology, BRIT, Mumbai, India). After treatment, the effects of both types of radiation on the oil samples were studied for determination of their reducing abilities and DPPH scavenging capacities.

Reducing power assay

The reducing power of the non-irradiated, UV- and γ-irradiated lavender samples (reduction of Fe^{3+} complex into the Fe^{2+}) were monitored by measuring the formation of Fe^{2+} at 700 nm according to the method described by Oyaizu (14) with modifications. Different quantities of oil (20 μL, 40 μL) were mixed with 200 μL of phosphate buffer (0.2 mol/L, pH 6.5) and 200 μL of potassium ferricyanide (1 %). Mixtures were incubated at 50 °C for 20 min in a water bath. After incubation, 200 μL of TCA (10 %, freshly prepared) were added to terminate the reaction. Samples were centrifuged at 6000 rpm, supernatants were taken out and 500 μL of distilled water and 0.1 mL of $FeCl_3$ solution (0.01 %) were added. The reaction mixture was left for 10 min. A higher absorbance of the reaction mixture indicated greater reducing power. For determination of the reducing power, a μ-QTM microplate spectrophotometer (Bio-Tek Instruments, Inc. Highland Park, Whinooski, VT, USA, India) was used. The absorbance was recorder at 700 nm and room temperature (22 °C to 25 °C). All experiments were performed in triplicate.

Electron paramagnetic resonance spectroscopy

For all EPR measurements an X-band EMX^{micro}, EPR spectrometer (Bruker, Germany) equipped with a standard resonator was used. Quartz capillaries were used as sample tubes. The capillary tubes were sealed and placed inside a standard EPR quartz tube (3 mm i.d., 150 mm length, 0.1 mm wall thickness) that was placed in the EPR cavity. All EPR experiments were performed in triplicate and repeated at room temperature (18 °C to 23 °C). Spectral processing (*g*-value calculation) was performed with Bruker WIN-EPR and SimFonia software.

Direct EPR spectroscopy of lavender oil samples before and after UV- or γ-irradiation

Direct EPR spectra of the lavender oil samples were recorded before and after UV- or γ-irradiation. The EPR settings for the non-irradiated and γ-irradiated samples were as follows: center field 3514.00 G, sweep width 200.00 G, microwave power 0.635 mW, modulation amplitude 10.00 G, gain 1×10^5, time constant 1310.72 ms, sweep time 133.12 s, 1 scan per sample. The EPR settings for the UV-irradiated samples were the same, only the modulation amplitude was reduced to 1.00 G.

DPPH radical scavenging capacity

The capacity for scavenging the DPPH radical was studied according to Bernardo dos Santos et al. (5) with slight modifications. Lavender oil (20 μL or 40 μL) was added to 250 μL of stock ethanol solution of DPPH (80 μmol/L). After stirring, the mixtures were incubated for 10 min and immediately transferred into the quartz capillaries and placed in the EPR cavity. The control samples contained 250 μL of stock ethanol solution of DPPH plus 20 μL or 40 μL of ethanol.

The DPPH radical scavenging capacities of the tested samples were calculated according to the equation:

$$Scavenged\ DPPH\ radical\ (\%) = [(I_0 - I)/I_0] \times 100\ \%,$$

where I_0 is the integral intensity of the DPPH signal of the control sample and I is the integral intensity of the DPPH signal after addition of the tested oil sample to the control sample. The EPR settings were as follows: center field 3516.00 G, sweep width 200.00 G, microwave power 3.232 mW, modulation amplitude 5.00 G, receiver gain 5.02×10^3, time constant 163.84 ms, 1 scan per sample.

Statistical analysis

Statistical analysis was performed with Statistica 6.1, StaSoft Inc., and results were expressed as means ± standard error (SE). Statistical significance was determined by Student's *t*-test. Value of $p < 0.05$ were considered statistically significant.

Results and Discussion

The effects of different types of radiation on the structure, reducing power ability and radical scavenging capacity of Bulgarian lavender oil are presented in **Fig. 1**, **Fig. 2** and **Fig. 3**. The reducing abilities of the UV-irradiated samples (0.336 ± 0.0121, for 20 µL; and 0.450 ± 0.019, for 40 µL) and γ-irradiated samples with a dose of 5 Gy (0.410 ± 0.0147, for 20 µL; and 0.427 ± 0.0251, for 40 µL) of the lavender oil, were significantly increased compared to those of the non-irradiated samples (0.2430 ± 0.0181, for 20 µL; and 0.268 ± 0.0244, for 40 µL) (**Fig. 1**). As seen, when the radiation doses increased, the reducing abilities of the γ-irradiated samples decreased: 0.410 ± 0.0147 v.s 0.223 ± 0.0112, for 20 µL; and 0.427 ± 0.0251 vs. 0.253 ± 0.0175, for 40 µL; at 5 Gy and 30 Gy radiation dose, respectively. Moreover, the highest reducing activity was found for the samples that received 5 Gy as compared to the non-irradiated oil samples.

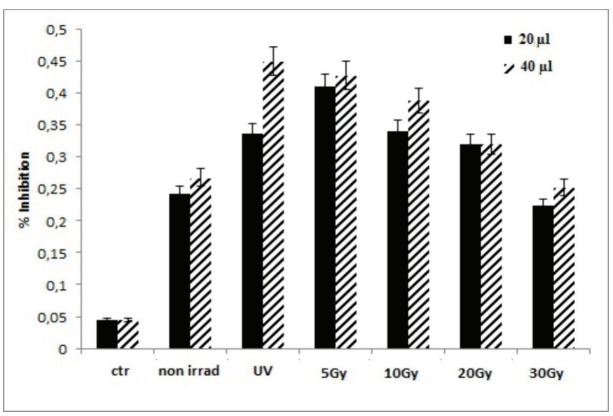

Fig. 1. Reducing power of lavender oil (20 µL, 40 µL) before and after UV- and γ-irradiation.

The EPR spectra recorded in the lavender oil before and after UV- or γ-irradiation are presented in **Fig. 2**. A single, almost symmetrical, stable EPR signal with a *g*-value of 2.0047 ± 0.0002 was registered in the non-irradiated oil sample (**Fig. 2A**). For the UV-irradiated sample, a single EPR line

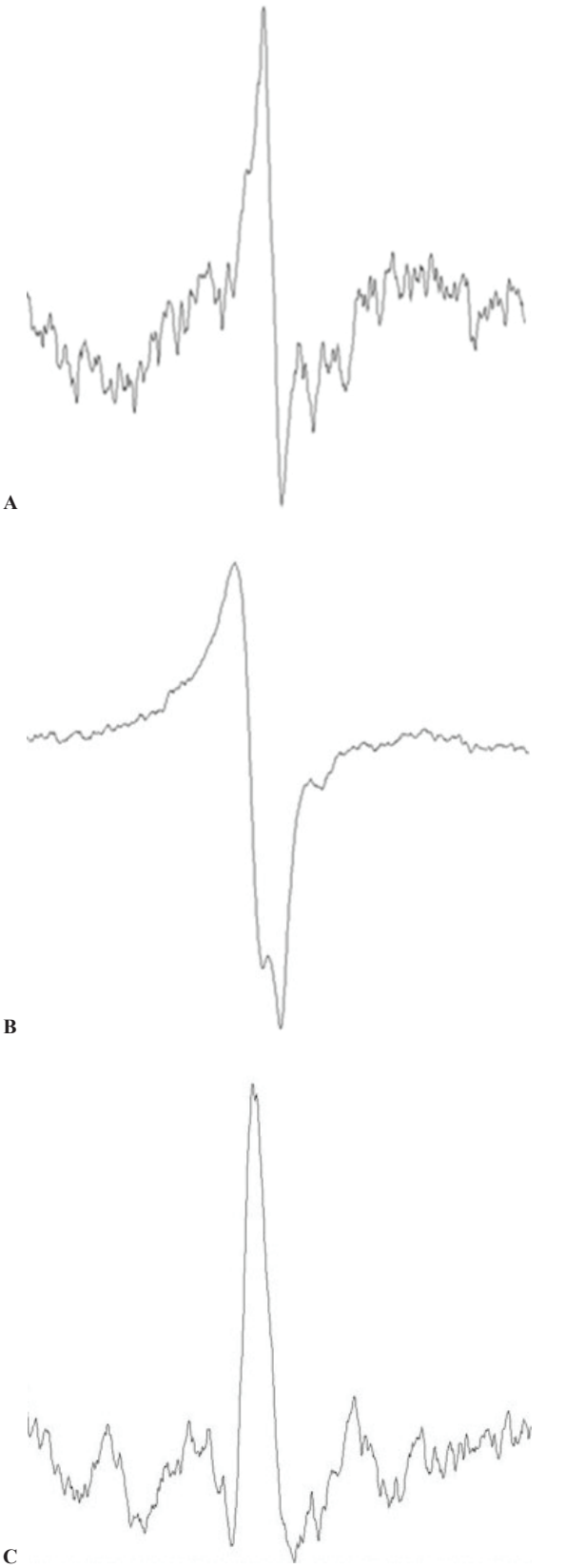

Fig. 2. EPR spectra of Bulgarian lavender oil before irradiation (**A**), and after UV-irradiation (**B**) and γ-irradiation, 20 Gy (**C**).

(g = 2.01050 ± 0.00005) with a small characteristic splitting (g = 2.0048 ± 0.0002) was registered (**Fig. 2B**). Some difference was observed in the shape of the EPR signal of non-irradiated and UV-irradiated samples (**Fig. 2A** and **Fig. 2B**). It should be mentioned that the strong EPR signal with a g-value of 2.0047 of the non-irradiated sample (**Fig. 2A**) after UV-irradiation almost disappeared and is visible only as a small characteristic splitting in the UV-irradiated sample (g = 2.0048, **Fig. 2B**). Based on this finding, we suppose formation of a new radical structure in the oil sample as a result of UV-irradiation.

In all γ-irradiated samples a single EPR signal was also recorded with a g-value of 2.0017 ± 0.0002 (**Fig. 2C**). The lower g-value of the γ-irradiated oil samples might be explained with the arising of a new radical structure caused by γ-irradiation. The intensities of the EPR signals registered in the UV- and γ-irradiated samples were considerably higher than those of the non-irradiated oil samples (data not shown). Moreover, 22 and 60 days after the irradiation treatment, the same EPR spectra with almost the same intensities were registered in the UV- and γ-irradiated oil samples, indicating that the radical structures formed after UV- and γ-irradiation in the studied Bulgarian lavender oil were quite stable. Since the g-values and shapes of the EPR signals scored in the UV- and the γ-irradiated samples were different from those registered in the non-irradiated samples, it could be assumed that the free radical structure initially present in non-irradiated Bulgarian lavender oil was affected by the UV- and γ-treatment.

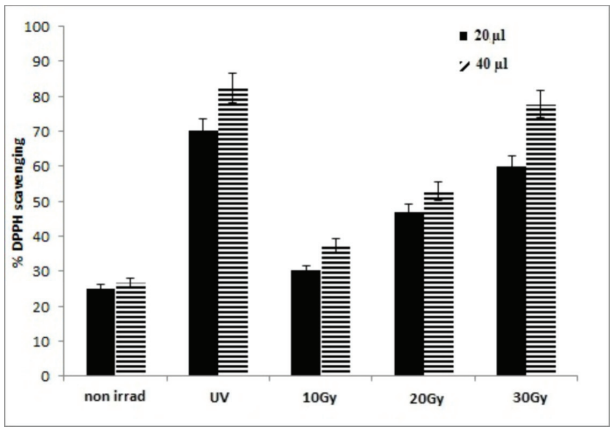

Fig. 3. Percent DPPH radicals scavenged by lavender oil samples (20 µL, 40 µL) before and after UV- and γ-irradiation.

The DPPH test is easy to perform, reliable and reproducible, and for this reason has been widely used in the analysis of antioxidant activities, both in the techniques based on measurement of the optical absorption intensity at 515 nm to 517 nm and in EPR spectroscopy. The fact that the EPR spectrum of DPPH has a relatively simple signal solely due to the free radical, is an advantage in optical spectroscopy (27). Based on this fact, we studied the radical scavenging capacity of Bulgarian lavender oil by means of EPR spectroscopy. The results from the determination of the DPPH radical scavenging capacity in lavender oil before and after UV- and γ-irradiation are shown in **Fig. 3**. It was obtained that, when the γ-irradiation doses increased, the percent of the scavenged DPPH radicals increased statistically significantly ($p < 0.05$). The maximum scavenging capacity (77.67 %) for the lavender oil (40 µL) was observed at a dose of 30 Gy radiation, which is about three-fold higher than the DPPH scavenging capacity of the non-irradiated samples (26.79 %, 40 µL).

The same tendency was demonstrated for the UV-irradiated samples. Moreover, the UV-irradiated samples (40 µL) showed more than three-fold higher DPPH scavenging capacity (82.31 %) in comparison with that of the non-irradiated samples (26.79 %, 40 µL) (**Fig. 3**). As a whole, the UV-treated samples showed higher scavenging capacity towards DPPH, in comparison with γ-irradiated samples. This difference in the DPPH radical scavenging capacity might be explained by formation of different stable free-radical structures after UV- and γ-irradiation of the lavender oil (see **Fig. 2B** and **Fig. 2C**).

Conclusions

In the present study, the effect of UV- and γ-radiation on Bulgarian lavender oil isolated from *Lavandula angustifolia* Mill. was investigated, to the best of our knowledge, for the first time. By means of EPR spectroscopy, the UV- and γ-irradiated oil samples were found to show excellent DPPH radical scavenging capacity in comparison with that of the non-irradiated oil samples. Based on these preliminary results, it could be suggested that, after proper UV- or γ-irradiation treatment, Bulgarian lavender oil might find application as a good radioprotector and antioxidant in cosmetics and medicine.

Acknowledgements

This study was supported by grants of the Ministry of Education, Youth and Science (Indo-Bulgarian collaborative project, BIn-7/2008), and by the European Social Fund through Operative Programme "Human Resources Development" 2007–2013, grant No. BG051PO001-3.3.06-006 – "Support for development of PhD students, post doctorals, post-graduate students and young scientists.

REFERENCES

1. **Anderson C., Lis-Balchin M., Kirk-Smith M.** (2000) Phytother. Res., **14**, 452-456.
2. **Arora R.** (2008) Homeland Defense and Space, CABI Publishing, Wallingford, Oxon, UK, p. 332.
3. **Arora R., Gupta D., Chawla R.** (2005) Phytother. Res., **71**, 425-435.
4. **Basch E., Foppa I., Liebowitz R.** (2004) Journal of Herbal Pharmacotherapy, **4**(2), 63-78.
5. **Bernardo Dos Santos A., Silva D.H.S., Bolzani V.S., Santos L.A., Schmidt T.M., Baffa O.** (2009) J. Braz. Chem. Soc., **20**(8), 1483-1492.
6. **Datta K., Sinha S., Chattopadhyay P.** (2000) Natl. Med. J. India, **13**(6), 304-310.
7. **Di Sotto A, Mazzanti G, Carbone F.** (2011) Environ. Mol. Mutagen., **52**(1), 69-71.

8. **Directive 1999/3/EC of the EP** (1999) Official Journal of the European Communities L, **66**, 24-25.
9. **Georgiev E., Stoianova A.** (2002) Essential Oils, Perfumery and Cosmetics, **14**, 47-50. (In Bulgarian)
10. **Georgiev E.** (1995) Technology of Natural and Synthetic Aromatic Products, Zemizdat, Sofia. (In Bulgarian)
11. **Halliwell B., Gutteridge J.M.C.** (1990) Methods Enzymol., **186**, 1-85.
12. **Kose E., Sarsilmaz M., Meydan S.** (2011) European Revue Medical Pharmacology Science, **15**(5), 538-542.
13. **Matthaus B.** (2002) J. Agr. Food Chem., **50**(12), 3444-3452.
14. **Oyaizu M.** (1986) Nippon Shokuhin Kogyo Gakkai-Shi., **35**, 771-775.
15. **Paul P., Unnikkrishnan M.K., Nagappa A.N.** (2011) Indian Journal of National Products and Resource, **2**(2), 137-150.
16. **Polovka M., Brezova V., Stasko A.** (2003) Biophys. Chem., **106**, 39-56.
17. **Perez M.B., Calderon N.L., Croci C.A.** (2007) Food Chem., **104**, 585-592.
18. **Raffi J., Yordanov N.D., Chabane S.** (2000) Spectrochimica Acta A, **56**, 409-416.
19. **Soković M., Glamočlija J., Marin P.D.** (2010) Molecules, **15**(11), 7532-7546.
20. **Sen S., Chakraborty R., Sridhar C., Reddy Y.S.R., De B.** (2010) International Journal of Pharmaceutical Sciences Review and Research, **3**(1), 91-100.
21. **Shaw D., Annett J.M., Doherty B.** (2007) Phytomedicine, **14**(9), 613-620.
22. **Suhaj M., Horvathova J.** (2007) J. Food Nutr. Res., **46**(3), 112-122.
23. **Suhaj M.** (2006) J. Food Compos. Anal., **19**, 531-537.
24. **Stanev S.** (2008) Bernburger Winterseminar, Fachtagung Arznei– und Gewürzpflanzen, 18–21 Febr., Bernburg, 46-47.
25. **Stanev S.** (2006) In: Proceedings of the International Scientific Conference, 1–2 June, Stara Zagora, Bulgaria, 169-172.
26. **Wettasinghe M., Shahidi F.** (2000) Food Chem., **70**(1), 17-26.
27. **Yordanov N.D., Christova A.G.** (1997) Fresen. J. Anal. Chem., **358**, 610.