Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 101 -107) 2019

Study of the radical-scavenging activities and radioprotective properties of Bulgarian essential rose oil from *Rosa Damascena Mill*

Yanka D. Karamalakova[1], Galina D. Nikolova[1], Natasha Kovacheva[2], Antoaneta M. Zheleva[1], Veselina G. Gadjeva[1]*

[1]*Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora, Bulgaria*

[2]*Agricultural Academy, Institute of Roses and Aromatic Plants, Kazanluk, Bulgaria*

Received March 23, 2019; Revised April 5, 2019

The present study for the first time reported radical-scavenging activities and radioprotective properties of Bulgarian essential rose oil from Rosa Damascena Mill. The chemically pure rose oil (100 %) with GC- established oil composition was tested non-irradiated and after UV-B, gamma (γ) radiation at doses 2.5, 5, 10, 20 and 30 Gy. By direct EPR spectroscopy method, the presence of stable free radical structures was established either in non-irradiated oil and in UV-B and γ- irradiated samples. It should be pointed out that stable free radical structures were registered in the same oil samples 2 months after the irradiation. Furthermore, as before and after UV-B/γ irradiation oil showed well expressed radical-scavenging activity and radioprotective properties against reactive oxygen such as superoxide radicals ($\bullet O_2^-$), hydroxyl radicals (•OH) and against DPPH stable radical. Current results characterize the rose oil as a promising source of natural substances that have radical-scavenging activity and radiation protective properties, and in future could be suitable for the development of new medications and dietary supplements.

Key words: Rosa Damascena Mill., EPR spectroscopy, radical-scavenging activity, Radioprotection

INTRODUCTION

Widely available evidence suggests that exposure to ionizing radiation leads to significant changes in biological systems caused by the free radicals level increase. The free radical reactions are required for the normal body metabolisms, but at a certain threshold of action, they can harm the health, especially resulting from various diseases such as diabetes, neurodegenerative diseases [1], and immunosuppression. Radiation-induced reactive oxygen species (ROS), e.g. hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), superoxide anions ($\bullet O_2^-$) and singlet oxygen species ($^1O_2$) and peroxynitrite ($ONOO^-$), are reliably linked to the site of their generation or detoxification by endogenous antioxidant protection in order to maintain the optimal cellular function. Radiation exposure and various pathological conditions are able to generate and increase the levels of generated oxygen species, which are not utilized by body detoxification mechanisms [2]. Antioxidants are compounds that are able to retard or prevent the oxidation processes, to inhibit the effect of oxidation reaction caused by radiation-induced free radicals, by preventing or delaying damage to the system6. Moreover, typical mechanisms of natural antioxidants action are scavenging the ROS and nitrogen radicals (NO), decreasing the oxygen concentration by reducing the molecular oxygen oxidation potential, chelating the metals to prevent the free radical generation, and metabolizing the lipid peroxides to non-radical products. Recent reports demonstrated the usefulness of flower extracts diversity and their bioactive principles in *in vitro*/ *in vivo* model systems as radioprotective antioxidants that can effectively include an unbalanced redox status and disable radiation-induced oxidative stress [3].

The *Rosa Damascena Mill* (Trigintipetala Dieck, genus Rosa, Rosaceae family, Bulgarian rose) is a unique plant species for Western Europe [4] and is used as an integral part of traditional homeopathic medicine and pharmaceuticals. Many studies have reported Rose oil neuropharmacological effects, relaxant effects, an antibacterial, an antioxidant, an analgesic and an anti-inflammatory affects and an anti-HIV activity [5]. The genotype of cultivated Damascus Rose (low acute toxicity) is an ingredient in the composition and quality of the rose oil. Bulgarian rose oil quality meets the standard (ISO 9842:2003, www. iso. org) [6]. Bulgaria is known for producing high-quality essential rose concrete and rose absolute from dry leaf species [7]. Extracted pure leaves oil form is the mixture of over 300 components belonging to the terpene and non-terpene hydrocarbons, glycosides, flavonoids, citronellol, geraniol, farnesol, alcohols, nerol, linalool and esters [8]. The gamma radiation 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

* To whom all correspondence should be sent:
E-mail: vgadjeva@mf.uni-sz.bg

Community list of food and food ingredients that may be treated with ionizing radiation and maximum overall average absorbed dose may be 10 kGy for aromatic herbs, spices and vegetable seasonings (Directive 1999/3/EC) [9]. There is minimal information about the effects of UV-B and γ-irradiation on antioxidant activity and the protective effects of herbs and essential flower oils. Essential oils extracted from γ-irradiated fruits were more effective as antioxidants. In addition, there were significant differences between oil and irradiated samples [10].

Electron paramagnetic resonance (EPR) identified different organic, inorganic, and transition metal radical species, changes in chemical composition and different spectrum shapes as a function of time and temperature or radiation type. Stability of the oxidative changes in oils may be evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (based on mechanism of single electron transfer) adopted for determining the antiradical activity of the hydrophilic and lipophilic antioxidants and oxidative stability of the oils [11].

This study purpose was to search for new substances of natural origin that could be used to develop effective radioprotective agents useful in medicine and cosmetics. In this respect were studied the effects of UV-B and γ- radiation on structural, antioxidant, and radical-scavenging abilities of rose oil by means of *in vitro* EPR and spectrophotometric technique.

EXPERIMENTAL

Plant material and Clevenger water-steam distillation: The flowers were picked up from the rose plantation, located near IREMK, Kazanlak, Bulgaria. The collection of flowers starts at stage IV-V (7 a.m.), at a relative humidity 86-93%, temperature 13.5-14.8°C [12]. The processing of raw floral material begins immediately by hydro-distillation for 2.4h with Clevenger apparatus with distillation parameters: water -floral material- (4:1 ratio), distillate T°C 25÷30°C.

***GC-Analysis**:* The components of the essential oil were identified by gas chromatography (GC) instrument (GC- PYE UNICAM) equipped with a flame ionization detector (FID) and EKONO – CAPTM ECTM cross–linked fused silica capillary column (1.30m long x 0.32mm TM internal diameter). The injector and detector (FID) temperatures were maintained at 300°C and 300°C, respectively. Hydrogen was the carrier gas at velocity winner 1.3 mL/min and injection volume at 0.1μL/min. Chromatograms obtained to identify representative and distinctive ingredients given in BS ISO 9842– 2004. For the accuracy of the results, pure substances (witnesses) were used for the determination of the peaks. The identification/percentage of the constituents was carried out against the retention time and calculated by electronic integration of FID peak areas, without correction factor.

Irradiation: The oil samples (in de-aerated capillary) were irradiated with UV-B (UV-vis Transilluminator- 4000, Bulgaria; 290-320nm; two lamps; 220V~50Hz; microwave power 7.70VA) at 2h rate and relative humidity- 40%. Radiation dose (2.5; 5; 10; 20; 30 Gy) was delivered from a 60Co γ- chamber (Gamma Cell 5000, Board of Radiation and Isotope Technology, India) at a dose rate of 1.4 Gy/h. Dosimeter was carried out using Baldwin Farmer's secondary dosimeter and Fricke's Chemical Method.

In vitro direct EPR studies on non-irradiated rose oil and UV or γ-irradiated: EPR experiments were carried out an X-band- EMX micro spectrometer (Bruker, Germany) equipped with the standard resonator. Spectral processing (g-value calculation) was performed with Bruker WIN-EPR and Sim-Fonia software.

***EPR determination of antioxidant activity**:* The ability to scavenge DPPH stable radical was studied according to Santos et al. [13]. Non-irradiated Rose oil (50μg/mL), and UV or γ-irradiation was added to 250μL ethanol solution of DPPH (80μmol/L). After incubation at 23°C for 10 min dark and transferred into the EPR cavity, the scavenging ability was calculated as follows:

$$\text{Scavenged DPPH radicals (\%)} = [(I_0 - I)/I_0] \times 100,$$

where $\mathbf{I_0}$ 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 control samples contained 250μL DPPH/ ethanol solution + 50μl ethanol.

SPECTROPHOTOMETRIC METHODS

***Hydroxyl radical scavenging potential estimation**:* The generation and detection of non-site-specific hydroxyl radicals (•OH) were carried out by Halliwell et al. [14]. After 55ºC incubation for 15 min/dark, the pink reactive chromogen from deoxyribose degraded by •OH was measured at 532 nm. The scavenging potential against hydroxyl radicals (%) was calculated as follows:

$$\text{\% Inhibition} = (A_{control} - A_{test}) / A_{control} \times 100$$

***Superoxide radical scavenging ability**:* The superoxide scavenging ability (SSA) of γ-irradiated and rose oil alone (0.1– 2.5μL/ml) was determined by using the nitroblue-tetrazolium reduction [15]. After 10 min incubation at 24°C, the

n-butanol layer was separated by centrifugation (1000 x g) and intensity of chromogen was measured at 560 nm.

Statistical analysis*:* All experiments were carried out in triplicate and repeated at 23°C. 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. The value of $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

It is well documented that ionizing radiation exposure induced ROS. Higher doses of radiation could lead to DNA fragmentation and lesions, membranes oxidation and denaturation of functional proteins and biomolecules [16]. An abundance of herbal extracts and aromatic herb oils is extensively screened to minimize ionizing radiation-induced deterministic effects developed after radiotherapy and free radicals generation during radiation exposure [17]. Herbal extracts have evolved an array of phytochemicals with radioprotective effects, possibly due to free radicals scavenging, the DNA repair processes enhancement, anti-inflammatory and antimutagenic. Essential oils isolated from different plants and exhibiting antioxidant activity are of great interest because may preserve from the toxic effects of oxidants [18].

Previous studies showed evidence that suggested diseases such as brain dysfunction; heart disease, cancer and immune system decline might be a result of cell damage from ROS and RNS. Essential oils capable of scavenge ROS/RNS can also play an important role in the prevention of different diseases [19]. Search for natural protectors against UV and γ- radiation directed us, to study the UV and γ- irradiation effects on antioxidant and free radical scavenging properties of Bulgarian rose oil by spectrophotometric and EPR technique. The rose oil was analysed by GC and the results were summarized in Table1. GC-analysis afforded 14 components representing 90.88% of the total identified composition. The light yellow oil was collected in the graduated tube/stored at 40°C in the dark for period of 15 days: density 0.854 g/cm^3/20°C; refraction number 1.462 and acid number less than 4.0, has been deposited at the Medical Faculty, Stara Zagora, Bulgaria.

In our study using direct EPR were registered stable radical structures both before and after UV and γ- irradiation of rose oil (Fig. 1). We assume that as a result of partial rose oil oxidation, some of its constituents especially eugenol might form radicals stabilized by resonance due to conjugation of the *o*-methoxy group and the aromatic ring with the double bond belonging to a propanoid chain of eugenol. Since the EPR spectrum in non-irradiated rose oil sample was identical with formerly reported EPR spectrum of the semiquinone radical registered in eugenol solution [20], we accept that the radical structure recorded in non- irradiated oil was also semiquinone derived from eugenol oxidation. It is well-established fact that UV and visible light accelerate autoxidation processes, by triggering the hydrogen abstraction, resulting in the radicals' formation [21]. Probably UV and gamma irradiation of our rose oil samples provokes auto-oxidation of the eugenol causing increased formation of dihydroeugenol and further oxidation of the later generates stable radical structures confirmed by EPR. Direct spectra recorded in non-irradiated rose oil and after UV- and γ- (2.5Gy) irradiations are presented in Fig. 1. Almost symmetrical single EPR spectral line with the g-value 2.0048 ± 0.0002 was registered in non-irradiated rose oil.

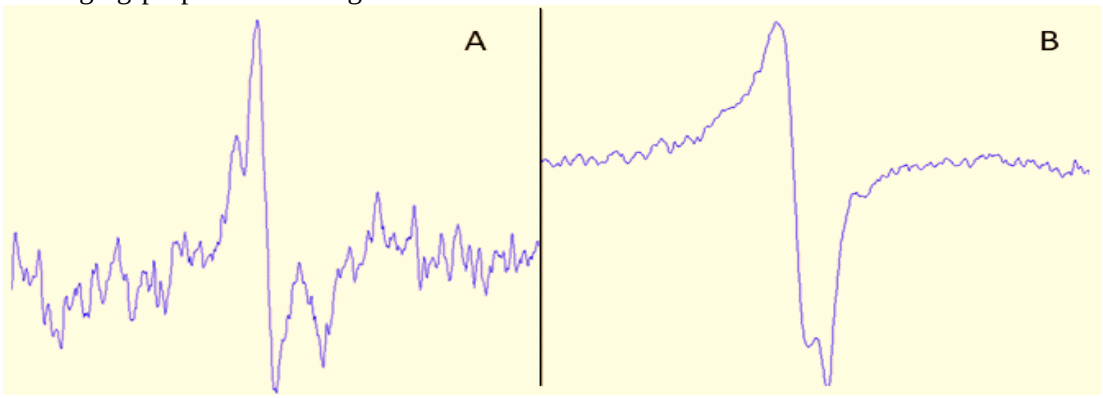
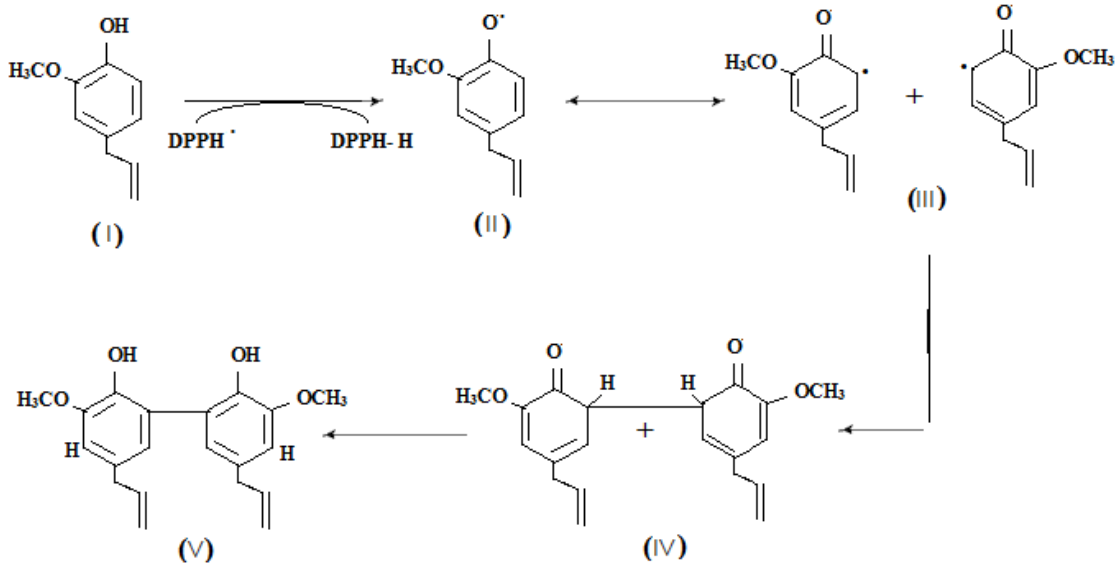


Figure 1. Direct EPR spectra recorded of non-irradiated (A) and UV-B/γ- irradiated (2.5Gy) rose oil (B).

The *in vitro* DPPH radical scavenging activity of non-irradiated rose oil at 50μg/mL concentration and UV and γ- irradiated is presented in Fig. 2. Before irradiation the maximal DPPH activity was 42.07 ± 1.22%, while, UV and γ- irradiated samples up to 10 Gy showed statistically significant higher

levels of DPPH scavenging, comparing to non-irradiated rose oil (85.1 ± 2.14%, 87.4 ± 2.67 and 86.42 ± 2.14%, t-test). Upon irradiation range higher than 10Gy (at 20Gy and 30Gy) was observed a considerable decrease in scavenging activity. After UV or 2.5Gy irradiation were registered spectra consisting of well expressed strong spectral line with g-value 2.01050 ± 0.0005 and with a small characteristic splitting. We believe the higher quenching abilities of the irradiated rose oil not also against DPPH (Fig. 2) but against ROS/RNS demonstrated here might due to the generation of dihydroeugenol during γ-irradiation (Scheme 1).



Scheme 1 One of the proposed mechanisms of interaction between eugenol and DPPH radical and formation of dehydrodieugenol (V), according to Bortolomeazzi et al [36].

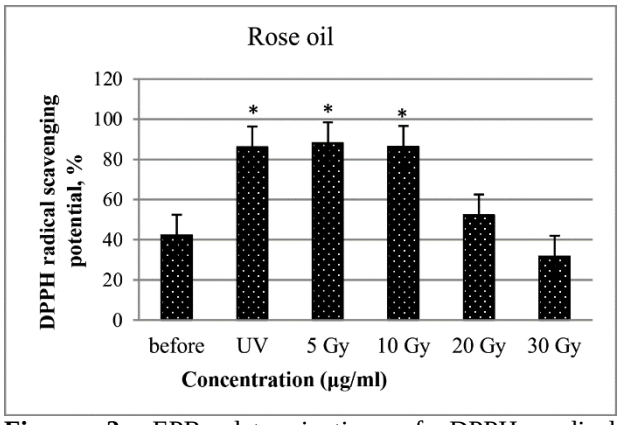


Figure 2. EPR determination of DPPH radical scavenging potential in the non-irradiated samples and UV-B/ γ- irradiated mixtures containing 50 mg/mL of rose oil. Each experiment was performed in triplicate and was repeated three times. The results are expressed as mean ± s.d.: *p< 0.05 compared to the non-irradiated rose oil.

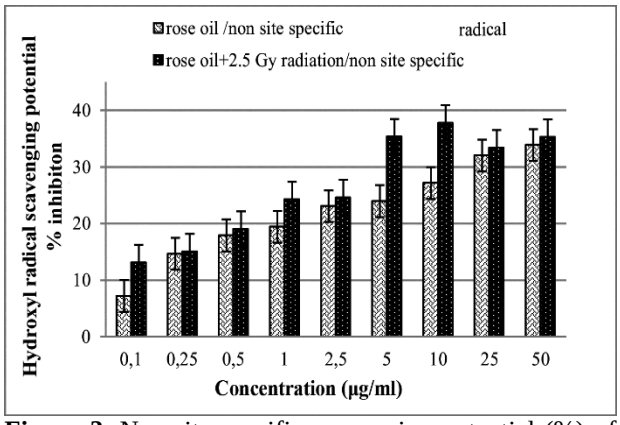


Figure 3. Non-site-specific scavenging potential (%) of hydroxyl radical (•OH) in concentration range (0.1 – 50µg/mL) of non-irradiated and γ- irradiated (2.5Gy) rose oil.

This supposition is indirectly confirmed by registration of different EPR spectra before and after γ-irradiation of the rose oil. It has to be emphasized, that when the dose of γ- irradiation was increased the radical scavenging abilities of rose oil significantly decreased (Fig. 2).

The considerable decrease in radical scavenging ability of the rose oil at higher doses (>5Gy) of γ-irradiation might explained with significant structural changes in some constituents as geraniol, citronellol, and eugenol, which probably largely determine rose oil antioxidant activity. Current in vitro experiments on non- and irradiated Bulgarian rose oil marked it as a potential antioxidant that might be used for reducing damaging effects of ROS induced by UV and gamma radiation

The hydroxyl radical-scavenging potential of the rose oil is shown in Fig. 3. Results demonstrated an inhibition in a dose-dependent manner, for both samples. Moreover, the maximal % of inhibition for non-irradiated rose oil was 32.82 ± 1.21 for

50μg/mL, while this percent for the irradiated sample was 36.95 ± 2.77, for 10μg/mL. The •OH radicals generated by the reduction of H_2O_2 and different radiation, contribute significantly for damaging different cellular components by lipid peroxidation (LPO), protein damage and membrane destruction. As is seen the highest quenching abilities towards •OH was found for 2.5Gy irradiated sample at a concentration of 10μg/mL, which means the γ- irradiated rose oil probably is directly involved in preventing of propagation of lipid peroxidation process in the tested system and might be a better scavenger for ROS, as compared to non-irradiated sample (Fig. 3) [22]. The superoxide radical scavenging ability results of 2.5Gy- irradiated and non-irradiated rose oil are presented in Figure 4. In the studied concentrations range (0.1- 2.5μg/mL) a dose dependent increase in the SSA was found either for non-irradiated rose oil or γ-irradiated samples. It must be emphasized that scavenging of superoxide radical by rose oil and 2.5Gy irradiated rose oil was comparable to that of quercetin, used as a positive control (*result not shown*). The highest SSA was achieved at the highest tested concentration (2.5μg/mL) for both non-irradiated and 2.5 Gy- irradiated samples (16.18 ± 1.3 % and 17.98 ± 1.74 %, t-test). Radical scavenging abilities of non-irradiated, and UV and γ- irradiated rose oil demonstrated by the methods used in this study can be explained by the presence of various constituents. Volatile compounds belonging to different classes of organic compounds have been isolated and identified in oil from *Rose Damascena* Mill. [23]. Components identified in rose oil include monoterpene alcohols (citronellol, geraniol, linalool, nerol and farnesol), hydrocarbons (nonadecane, eicosane heneicosane), alcohols, esters, ethers. The Ulusoy *et. al.* [24] has evaluated the chemical compositions of essential rose oil isolated from *Rose Damascena* Mill. by GC-MS and found the main constituents were citronellol and geraniol (>55%). Nevertheless, phenylpropanoid such as eugenol is also an important component in rose essential oil. It is well documented that the composition of Bulgarian rose oil is of low variability [25] and citronellol and geraniol are constituents with the highest content in it (Table 1), and a certain amount of eugenol is also presented, as well.

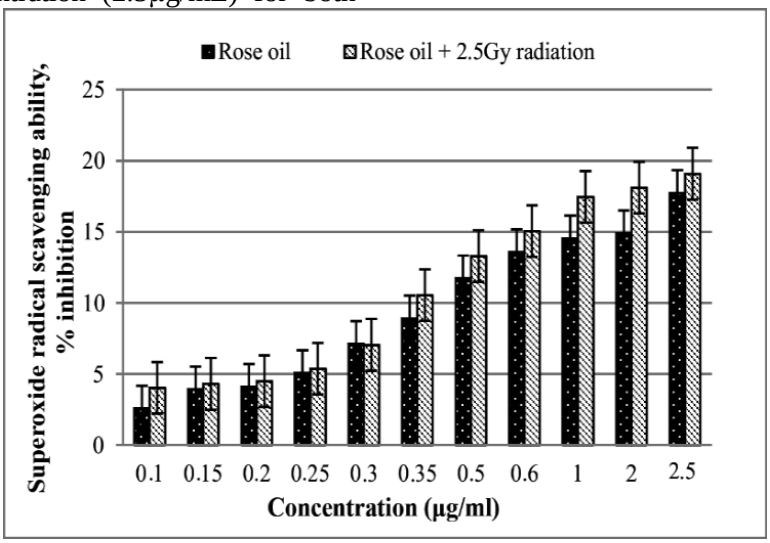


Figure 4. Superoxide radical scavenging ability of non-irradiated and γ- irradiated (2.5Gy) rose oil in concentration range (0.1 – 2.5μg/mL).

Antioxidant activity and radical scavenging abilities of constituents isolated from different essential oils including rose oil have been reported in a great number of studies. Formerly, Choi *et. al.* [26] identified geraniol and citronellol as potential antioxidants. Effectiveness in scavenging abilities towards DPPH radical of geraniol and citronellol was explained by their significant content in various essential oils48. Wei *et. al.* [27] was found that oil isolated from *Rose Damascene* Mill. exhibited well expressed DPPH radical scavenging activity and good inhibitory effect toward MDA formation from squalene after UV-B. The same authors considered that the main compound in rose oil with high antioxidant activity was citronellol (content 34.2%). Mileva *et. al.* [28] reported the good ability of geraniol to scavenge DPPH stable radicals and this ability is the reason for up-regulation of antioxidant mechanism and is close related with its cardioprotective effect [29]. Moreover, Stobieska [30] has studied the mechanism of geraniol interaction with DPPH radical and concluded the presence of allylic hydrogen atom (at the position 1C) close to the –OH group seemed to be essential for the observed geraniol anti-radical activity.

Table 1. The percentage of chemical composition of Bulgaria Essential rose oil from *Rosa Damascena Mill*

Compounds	(*Rosa Damascena* Mill) oil (x ± Sx)	BDS- ISO 9842 (min-max)
Citronellol + Nerol	31.05±2.34	25.0-46.0
Geraniol	20.6±1.37	15.0-22.0
Geranial	1.39±0.16	
Eugenol	1.38±0.18	
Methyl-eugenol	0.65±0.08	
ß-Phenyl-Ethanol	0.42±0.05	max 3,5
Linalool	1.77±0.08	
cis-rose oxide	0.39±0.01	
trans rose oxide	4.27±0.00	
β- Damastcenon	0.12±0.00	
Geranil-acetate	1.30±0.10	
Ethanol	0.04±0.00	max 2
Paraffins		
Heptadecan (C_{17})	2.93±0.36	1.0-2.5
Nonadecan (C_{19})	18.5±1.8	68.0-15
Heneykozan (C_{21})	3.27±0.02	3.0-5.5

Current study demonstrates a significantly increased scavenging capability against the DPPH radical for both UV and 5Gy irradiate rose oil samples. Considering that in physiological conditions the primary ROS ($\bullet O_2^-$) is a precursor in the generation of the highly toxic •OH, we also studied scavenging activity of non -irradiated and UV and gamma radiated rose oil against these both ROS. Results obtained indicate that irradiated rose oil ($p<0.01$) possess significantly higher superoxide anion radical-scavenging ability than non-irradiated samples (Fig. 4). The quenching ability of non-irradited and 2.5Gy–irradiated rose oil to superoxide radical anions seems to be directly related to the prevention of propagation of the lipid peroxidation process and seems to be a good ROS scavenger. We assume the well expressed •OH scavenging ability of the irradiated rose oil is able to assist successfully the antioxidant defense in neutralizing these highly toxic radicals. Based on the above reported findings and the present results we accept that citronellol, geraniol, and eugenol, in our rose oil samples are mainly responsible for good scavenging activity demonstrated towards stable DPPH, and unstable $\bullet O_2^-$, •OH.

Upon exposure to photochemical, biochemical or radiation chemical processes that involve oxidation of phenols or reduction of quinones might be formed phenoxyl or semiquinone radicals, correspondingly. In a number of previous studies was reported that the interaction of different radicals with phenols and quinones led to a generation of phenoxyl and semiquinone radicals [31]. Production of free radical species on oxidation of the volatile fraction of several plants possessing medicinal properties has been investigated by Deighton et al. [32]. By EPR spectroscopy they demonstrated the formation of stable free radicals upon reaction of essential oils isolated from oregano, summer savoury and thyme after UV irradiation and $\bullet O_2^-$ radical confirmed that the radical structures originated from oxidation of two main phenolic constituents presenting in the oils-carvacrol and thymol. It is known that phenols such as carvacrol, thymol, or the phenolic ether eugenol can disrupt or rather delay autoxidative chain reactions [33]. Antioxidant effectiveness of these compounds is based on the possibility for abstracting rapidly the phenolic hydrogen atom, resulting in formation of free, comparably long-lived radicals stabilized by isomerization to an alkyl-substituted tertiary position or electron-releasing groups. These antioxidants called primary are able to scavenge free reactive radicals such as alkyl, alkoxy, or peroxyl which leads to increasing the stability of the natural product containing them [34]. Using EPR, Fujisawa and co-workers [35], studied eugenol dissolved in buffer (0.1 M $NaHCO_3$–Na_2CO_3, pH=9), and demonstrated the presence of a stable semiquinone radical. Bortolomeazzi *et. al*. [36], investigated the products of a reaction between eugenol and DPPH stable radical and found the main reaction product was dihydrodieugenol [36]. Moreover, its dimer formed by combining carbon atoms belong to two individual eugenol molecules and, these both carbons are in ortho-position relative the phenolic hydroxyl group (Scheme 1, V). In addition, higher radical scavenging activity of dihydrodieugenol with respect of the parent eugenol was found and explained by the presence of two phenolic OH-groups and ortho- benzene ring that allowed an

extensive conjugation stabilizing the phenoxyl radical of the dimmer [37].

CONCLUSION

These preliminary results characterize the Bulgarian essential rose oil as a good scavenger of stable and unstable radical species, suggesting that can be used as a protector against UV and γ-induced oxidative toxicity under physiological conditions.

Acknowledgement. *This study was supported by a grant of Ministry of Education, Youth and Science – Indo-Bulgarian collaborative project (BIn-7/2008), PIRSES- GA- 2012- 316067/ 2013-2016 and scientific projects № 21/2014; № 35/2014; №1/2018. The authors gratefully acknowledge the following people for their assistance with this project-work: Manish Adhikari, Poonam Malhotra.*

Conflict of interest*. The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.*

REFERENCES

1. P. Singh, A. Bali, N. Singh, A. Jaggi *Korean J. Physiol Pharmacol.*, **18**, 1 (2014).
2. B. Henderson, A. Miller, *Radiation Research **108***, 196 (1986).
3. P. Paul, M. Unnikrishnan, A. Nagappa *Ind J. Nat Prod Res* **2**, 137 (2011).
4. P. Beales, T. Cairns, W. Duncan, W. Grant, K. Grapes, P. Harkness, K. Hughes, J. Mattock, D. Ruston *Randomhouse Australia Pty. Ltd*. *Sydney (*1998).
5. M. Boskabady, S. Kiani, H. Rakhshandah *J Ethnopharm* ,**106**, 377 (2006).
6. ISO 9842:2003 Oil of rose (Rosa × damascena Miller). Available online: <*www.iso.org*>2003.
7. K. Rusanov, N. Kovacheva, A. Atanassov, I. Atanassov *Floriculture Ornamental Biotech*, **3,** 14 (2009).
8. M. Boskabady, M. Shafei, Z. Saberi, S. Amini, *Iranian J. Basic Med Sci*, **14**, 295 (2011).
9. European Directive 1999/3/EC of the European Parliament and of the Council of 22 February 1999 on the establishment of a Community list of foods and food ingredients treated with ionising radiation. *Office J Europ Comm* L66, **13.3**, 24–25 (1999).
10. M. Suhaj, J.Horvathova *J Food Nutr Res*, **46**, 112 (2008).
11. A. Prescha, M. Grajzer, M. Dedyk, H. Grajeta *J Am Oil Chemists' Soc.*, **91**, 1291 (2014).
12. V. Staikov, R. Decheva, A. Balinova-Tsvetkova *Rivista Ital.*, **57**, 192 (1975).
13. A. Santos, D. Silva, V. Bolzani, L. Santos, T. Schmidt, O. Baffa *J Brazil Chem Soc.*, **20**, 1483 (2009).
14. B. Halliwell, J. Gutteridge, O. Aruoma *Analit Biochem.*, **165**, 215 (1987).
15. P. Kakkar, B. Das, P. Viswanathan *Ind J Biochem Biophys.*, **21**,130 (1984).
16. N. Gillard, S. Goffinont, C. Buré, M. Davidkova, J. Maurizot, M. Cadene, M.Spotheim-Maurizot, *Biochem J.*, **403**, 463 (2007).
17. S. Hosseinimehr *Drug Discov Today* **12**, 794 (2007).
18. D. Maestri, V. Nepote, A. Lamarque, J. Zygadlo, Phytochem: Advanc.in Res. Res. Signopost: Kerala, India. Pp. 105-135 (2006).
19. M. Miguel, *Molecules*, **15**, 9252 (2010).
20. S. Fujisawa, T. Atsumi, Y Kadoma, H. Sakagami *Toxicol.*, **177**, 39 (2002).
21. E. Choe, D. Min *Compreh. Reviews Food Sci. Food Safety*, **5**, 169 (2006).
22. İ. Gülçin, M. Elmastaş, H. Aboul-Enein *Arab J Chem.*, **5**, 489 (2012).
23. N. Koksal, H. Aslancan, S. Sadighazadi, E.Kafkas *Acta Sci Pol Hortorum Cultus* **14**, 105 (2015).
24. S. Ulusoy, G. Bosgelmez-Tmaz, H. Secilmis-Canbay Tocopherol, Carotene, *Current Microbiol.*, **59**, 554 (2009).
25. K. Rusanov, N. Kovacheva, M. Rusanova, I. Atanassov, *Industr Crops and Prod*., **37**, 6 (2012).
26. H. Choi, H. Song, H. Ukeda, M.Sawamura, *J. Agric. Food Chem*., **48,** 4156 (2000).
27. A. Wei, T. Shibamoto *Cutaneous Ocular Toxicol*., **26**, 227 (2007).
28. M. Mileva, I. Nikolova, N. Nikolova, L. Mukova, A. Georgieva, A. Dobreva, A. Galabov *Acta Microbiol Bulgar* , **31**, 48 (2015).
29. P. Subramani, T. Sasivarnam, G. Krishnan, I. Selvanathan, G. Mani, E. Ramasamy, D. Thiruvengadam, *Int. J Pharmaceut. Sci* ,**7**, 420 (2015).
30. A. Stobiecka *Flavour and Fragrance J*. **30**, 399 (2015).
31. P. Neta, J. Grodkowski *J Phys Chem Ref Data*, **34**, 109 (2005).
32. N. Deighton, S. Glidewell, *J Sci Food Agric.*, **63**, 221 (1993).
33. C. Turek, F.Stintzing *Compreh. Reviews Food Sci. Food Safety*, **12**, 40 (2013).
34. B. Nanditha, P.Prabhasankar *Critical Reviews Food Sci. Nutr.*, **49**, 1 (2009).
35. S.Fujisawa, T.Atsumi, Y. Kadoma, H. Sakagami, *Toxicol.* **177**, 39 (2002).
36. R. Bortolomeazzi, G. Verardo, A. Liessi, A. Callea, *Food Chem*., **118**, 256 (2010).
37. H. Ali, A. Abo-Shady, H. Eldeen, H. Soror, W. Shousha, O. Abdel-Barry, A. Saleh *Chem. Central J.*, **7**, 53 (2013).