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EVALUATION OF GENOTOXIC AND ANTIGENOTOXIC PROPERTIES OF ESSENTIAL OILS OF *SESELI RIGIDUM* WALDST. & KIT. (APIACEAE)

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Abstract: The essential oils of genus *Seseli* are known for their beneficial biological activities and could present novel targets in the development of safe and effective preparations of plant products. The objective was to test the essential oils of different parts of *Seseli rigidum* from two natural habitats for potential genotoxic and antigenotoxic activities against H_2O_2-induced DNA damage in human whole blood cells *in vitro*, by the comet assay. The essential oil analysis showed a high falcarinol content in oil from the root, while oils of the fruit and aerial parts contained α-pinene as the main compound. Genotoxicity was not detected at any of the concentrations of the essential oils from the three parts of the plant from localities I and II. Although the antioxidant activity (established by the FRAP and DPPH tests) of the investigated oils was low, all oils demonstrated a strong antigenotoxic effect against H_2O_2-induced damage post-treatment, when the oils were applied after the oxidant. Based on the lack of pretreatment activity and the post-treatment reduction in DNA damage, the antigenotoxic effect of *S. rigidum* essential oils was probably based on the stimulation of DNA repair mechanisms. Environmental conditions did not affect the antigenotoxic properties of the oils. In conclusion, our results revealed the antigenotoxic properties of *S. rigidum* essential oils and appropriate and safe doses with beneficial effects under the described conditions.

Key words: *Seseli rigidum*; essential oils; antioxidant; DNA damage; comet assay

INTRODUCTION

Genomic damage may lead to chronic diseases, cancer and premature ageing. Compared to other macromolecules, DNA is more sensitive to oxidative damage, making it a suitable marker for examination of the effects of oxidant-induced damage in the cell (Cooke et al., 2003). Reactive oxygen species (ROS) are constantly generated during normal cellular activity (Barzilai and Yamamoto, 2004). However, an imbalance between the generation of ROS and the antioxidant capacity of cells can lead to oxidative stress. ROS increase can cause severe damage to macromolecules, especially to DNA. When DNA damage in cell occurs, repair mechanisms are activated in order to prevent genomic instability (Polo and Jackson, 2011). Unrepaired DNA damage can be detected by comet assay (Anderson et al., 1994).

Recent research interests have been directed toward detecting and examining different natural products as sources of health improving agents (Masood et al., 2013). An ethnobotanically targeted approach is still being broadly applied in the discovery of natural products with potential pharmacological activities (Lewis and Elvin-Lewis, 1995).

Seseli rigidum Waldst. & Kit. (Apiaceae) is an herbaceous perennial plant native to the Balkan Peninsula (Ball, 1968). Species of the genus *Seseli* are used in traditional medicine as anti-inflammatory agents, and for their carminative, stomachic, anthelmintic and antibacterial properties (Küpeli et al., 2006; Gonçalves et al., 2012). The essential oil of species of the genus *Seseli* showed antibacterial, antifungal (Gonçalves et al., 2012; Milosavljević et al., 2007; Stojković et al., 2009; Skalicka-Wozniak et al., 2010) and moderate cytotoxic activity (Gonçalves et al., 2012; Shahabipour et al., 2012). Ethyl acetate extracts of *Seseli* species from Turkey exerted anti-inflammatory and antinociceptive activity (Küpeli et al., 2006). The essential oil of the root, aerial parts and fruit of *S. rigidum* showed antimicrobial effects against laboratory control strains of bacteria and methicillin-resistant strains of *Staphylococcus aureus* (MRSA) (Marčetić et al., 2012). Also, *S. rigidum* flower essential oil demonstrated moderate antimicrobial and low antioxidant effects (Stojković et al., 2009).

Despite the potential beneficial activities, there are no published reports on the genotoxicity or antigenotoxicity of *S. rigidum* essential oils. Since the use of essential oils in therapeutic approaches may require high doses, screening for potentially genotoxic properties is of great significance in order to determine the optimal concentration for safe use. In this work, the effect of different concentrations of plant essential oils in attenuating DNA damage induced by hydrogen peroxide in human whole blood (WB) cells. The comet assay, as a well-established and sensitive test was used to evaluate DNA damage (Anderson and McGregor, 1998).

The production of essential oil is strongly influenced by genotype, environmental factors and interactions between genotype and environment. Among the abiotic environmental factors, climate and soil type can strongly influence the terpene biosynthesis and composition of the essential oil. Subsequently, the biological activity can vary depending on the composition of the essential oils from the plants that grow under different environmental conditions (Figueiredo et al., 2008).

The aim of this investigation was to study the antioxidant potential, genotoxic and antigenotoxic activity of the essential oil of root, aerial parts and fruit of *S. rigidum* from two natural populations in Serbia.

MATERIALS AND METHODS

Plant material

Root and aerial parts were collected in June 2010 during the flowering of *Seseli rigidum* and fruits in September 2010, in the Brdjanska gorge (longitude 43.986, latitude 20.418, altitude 288 m a.s.l) in western Serbia, from a humid climate and serpentine soil (locality I), and in Golubac (longitude 44.660, latitude 21.671, altitude 100 m.a.s.l) in eastern Serbia, from a semi-arid climate and calcareous soil (locality II). Specimens were deposited at the Herbarium of the Faculty of Pharmacy in Belgrade (3240HFF, 3233 HFF). Essential oils were obtained from dried and ground plant material by hydrodistillation in a Clevenger-type apparatus according to procedure given in European Pharmacopoeia 7.0 (2011).

Essential oil analysis

The volatile constituents were determined by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS). GC analysis was performed on an Agilent 6890N GC system equipped with 5975 MSD and FID, using a HP-5 MS column (30 m × 0.25 mm, 0.25 μm film thickness). Essential oil was dissolved in ethanol (2% v/v), the injection volume was 2 μL; injector temperature was 200°C, with a 10:1 split ratio. Helium was the carrier gas and its flow rate was 1.0 mL/min (constant flow mode). The column temperature was linearly programmed in the range 60-280°C at a rate of 3°C/min and held at 280°C for 5 min. The transfer line was heated at 250°C. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in the *m*/*z* range 35-550. The retention indices were experimentally determined using *n*-alkanes (C_8-C_{20} and C_{21}-C_{40}) injected after the essential oil, under the same chromatographic conditions. The identification of the compounds was based on comparison of their

retention indices (RI), retention times (t_R) and mass spectra with those obtained from authentic samples and/or the NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software, Wiley libraries, Adams database and literature (Adams, 2001). Relative percentages of the identified compounds were computed from the GC/FID peak area.

Ferric reducing antioxidant power

The total antioxidant potentials of essential oils were determined using ferric reducing antioxidant power (FRAP) assay based on a reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) and formation of a blue complex (Fe^{2+}-2,4,6-tripyridyl-s-triazine; Fe^{2+}-TPTZ), which increases the absorption at 593 nm (Benzie and Strain, 1996; Szőllősi and Varga Szőllősi, 2002). The amount of reduced Fe^{2+}-TPTZ complex was calculated from an equation of regression line constructed from the absorbance of water solutions of $FeSO_4 \times 7\ H_2O$ (0.1-1.0 mM) under experimental conditions. Results are expressed as μmol Fe^{2+}/mg extracts. All measurements were performed in triplicate. The natural antioxidants rutin and ascorbic acid served as a control. All reagents were supplied by Sigma-Aldrich, St. Louis, MO, USA.

DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity is based on the reduction of the free radical DPPH• and the decrease of absorbance at 517 nm (Bondet, et al., 1997). The SC_{50} value, which is the concentration of the tested extract that reduces 50% of the free-radical concentration, was calculated as mg/mL from plot of logarithms of concentration versus probit of scavenging effect. All determinations were performed in triplicate. The natural antioxidants rutin and ascorbic acid were used as a control. All reagents were supplied by Sigma-Aldrich, St. Louis, MO, USA.

Subjects

Heparinized human whole blood samples were obtained by venipuncture from six healthy donors aged under 25 years. Subjects were non-smokers who reported no alcohol consumption, were receiving no therapy or medication, nor taking dietary supplements.

Study design

Essential oils were dissolved in ethanol and then diluted with the phosphate-buffered saline solution. Final concentrations of essential oils were chosen according to the range of concentrations previously found to be effective in antimicrobial assessment of *S. rigidum* studies (Marčetić et al., 2012). As the highest activity was obtained with root essential oil, it was studied at a wider range of concentrations (12.5, 25, 50 and 100 μg/mL). The activity of the essential oil of aerial parts and fruit were investigated at three concentrations, 25, 50 and 100 μg/mL.

In order to estimate the genotoxic potential of the essential oils of root, aerial parts and fruit at different concentrations we used the following protocol. Suspensions of WB cells embedded in agarose gel on slides were made and treated with a 50 μL solution of essential oils at the indicated concentrations for 30 min at 37°C to evaluate the oils' ability to cause DNA damage. The experiment was simultaneously done with samples treated only with the solvent phosphate-buffered saline (PBS, Torlak Institute of Immunology and Virology, Belgrade, Serbia) as negative control, and 50 μM hydrogen peroxide (H_20_2, CAS No. 7722-84-1, ZORKA Pharma, Šabac, Serbia) as positive control. Treatment with 50 μM H_2O_2 for 20 min on ice was the smallest concentrations that produced a consistently high level of DNA fragmentation in exposed cells as compared to the untreated controls.

To examine the genoprotective potential of *S. rigidum* essential oils on human WB cells against H_2O_2-induced oxidative damage *in vitro*, we performed the following pretreatment protocol. Namely, the WB cell preparations on slides were first incubated with 50 μL of essential oils at tested concentrations for 30 min at 37°C, then rinsed with PBS and subsequently treated with 60 μL of 50 μM H_2O_2 for 20 min on ice. For the assessment of the antigenotoxic effects of the oils at intervention level, post-treatment with the essential

oils was performed, where WB cells were first exposed to H_2O_2 and afterwards treated with the oils under the same conditions as before. After the last treatment, cells were washed once more with PBS and DNA damage was evaluated. All experiments were carried out in duplicate and repeated six times.

The single cell gel electrophoresis assay

The comet assay was performed essentially as described by Singh et al. (1988). The alkaline comet test was used to detect DNA strand breaks and alkali-labile sites; the extent of DNA migration indicated the degree of DNA damage in the cell. First, cell viability was determined by the trypan blue exclusion method, and was found to be above 90% (Anderson et al., 1994). After the cell viability check, 6 μl of whole blood was suspended in 0.67% low-melting-point agarose (Sigma-Aldrich, St. Louis, MO) and pipetted onto SuperFrost™ glass microscope slides precoated with a layer of 1% of normal-melting-point agarose (Sigma-Aldrich, St. Louis, MO), spread using a coverslip, and maintained for 5 min on ice to solidify. After gently removing the coverslips, the WB cell suspension in agarose gel on slides were treated with the oil solutions and H_2O_2 as described above. Following the treatments, the slides were rinsed with PBS and covered with the third layer of 0.5% low melting agarose and again allowed to solidify on ice for 5 min. After removal of the coverslips, the slides were placed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X100 and 10% dimethyl sulfoxide, pH 10 adjusted with NaOH) at 4°C overnight and afterwards subjected to electrophoresis and staining with ethidium bromide, as described by Singh et al. (1988). The comets were observed and analyzed using an Olympus X 50 microscope (Olympus Optical Co., GmbH Hamburg, Germany), equipped with a fluorescence recording device at 100× magnification. Evaluation of DNA damage was performed as described by Anderson et al. (1994). Namely, cells were graded visually into five categories corresponding to the following amount of DNA in the tail: (A) no damage, <5%; (B) low level damage, 5-20%; (C) medium level damage, 20-40%; (D) high level damage, 40-95%; (E) total damage, >95%.

Analysis was performed on a 100 randomly selected cells per subject (50 cells from each of 2 replicate slides). In order to obtain semiquantitative analysis of data, DNA damage was characterized as DNA migration greater than 5% (total number of damaged cells from comet classes B+C+D+E), and the mean value was calculated for all subjects.

Statistical analysis

Statistical analysis of the comet assay results was performed using one-way analysis of variance (ANOVA). Values are expressed as mean±standard error of the mean (SEM) for n=6. GraphPad Prism 5.0 software was used. A difference at $p<0.05$ was considered statistically significant.

RESULTS

Essential oil analysis and antioxidant activity of the oils

The essential oil of *S. rigidum* root is characterized by a high content of polyacetylene falcarinol (Marčetić et al., 2012; 2013). In samples of root essential oil from locality I (Brdjanska gorge), the content of falcarinol (88.8%) was higher than in oil from locality II (Golubac, 57.5%) (Table 1). The essential oil of *S. rigidum* aerial parts is mainly composed of the monoterpenes α-pinene, sabinene and limonene (Marčetić et al., 2012; Šavikin-Fodulović et al., 2006). In the essential oils of aerial parts α-pinene (57.4% locality I and 44.7% locality II), sabinene (5.5% locality I and 16.1% locality II) and limonene (6.7% locality I and 8.4% locality II) also dominated. In accordance with previous investigations (Marčetić et al., 2012), the monoterpenes α-pinene (23.3% locality I and 21.1% locality II), sabinene (12.9% locality I and 19.2% locality II) and sesquiterpene β-phellandrene (17.4% locality I and 12.6% locality II) were the main constituents in fruit oil.

The total antioxidant potential of the investigated essential oils was in the range 0-0.38 μmol Fe^{2+}/mg (Table 1). The essential oil of aerial parts and fruit from locality I and the essential oil of the fruit from

Table 1. Phytochemical analysis and antioxidant activities of essential oils.

	Locality I (Brdjanska gorge)			Locality II (Golubac)				
	Root[a]	Aerial p.[b]	Fruit[c]	Root	Aerial p.	Fruit		
Content (%)								
α-Pinene	0.2	57.4	23.3	4.3	44.7	21.1		
Sabinene	tr[d]	5.5	12.9	1.2	16.1	19.2		
Limonene	tr	6.7	tr	0.8	8.4	tr		
β-Phellandrene	-	tr	17.4	tr	tr	12.6		
Falcarinol	88.8	-	2.9	57.5	0.3	2.7		
							Rutin	AA[e]
FRAP[f]	0.38±0.03	0	0	0.31±0,05	0	0.20±0,0	5.90±0.01	10.41±0.07
DPPH[g]	2.00±0.13	27.60±0.97	18.01±1,81	2.68±0,95	10.75±1.21	5.47±0.64	0.003±0.000	0.004±0.000

[a] essential oil of root; [b] essential oil of aerial parts; [c] essential oil of fruit; [d] trace (<0.1%); [e] ascorbic acid; [f] μmol Fe^{2+}/mg; [g] SC_{50} concentration of the oil that scavenge 50% of DPPH radical (mg/mL)

the locality II did not show reducing ability. The total antioxidant potential of the essential oils of the root from the both localities (0.38±0.03 and 0.31±0.05 μmol Fe^{2+}/mg) and the oil of fruit from locality II (0.20±0.0 μmol Fe^{2+}/mg) was modest when compared with the control antioxidants rutin (5.90±0.01 μmol Fe^{2+}/mg) and ascorbic acid (10.41±0.07 μmol Fe^{2+}/mg).

In the DPPH assay, the reactivity of the potential antioxidant with stable, free DPPH radical is measured. The concentrations of essential oils that reduce 50% of the DPPH radical were from 2.00 to 27.60 mg/mL (Table 1). The best DPPH-scavenging activity was obtained with the essential oil of root from both localities (2.00±0.13 and 2.68±0.95 mg/mL). Essential oils of aerial parts and fruit (10.75±1.21 and 5.47±0.64 mg/mL, respectively) from locality II showed a more pronounced DPPH-scavenging ability than the oil from locality I, but nonetheless moderate activity in comparison with the control antioxidants rutin (0.003±0.000 mg/mL) and ascorbic acid (0.004±0.000 mg/mL).

Evaluation of genotoxic activity of essential oils

The comet assay was employed to assess the ability of essential oils of *S. rigidum* to cause DNA damage, visible as DNA migration. The analyzed results demonstrated that the concentrations (12.5-100 μg/mL) of root essential oil from localities I and II did not significantly increase the total number of DNA-damaged cells in regard to the control treated only with PBS, which clearly shows that the tested essential oils did not display a genotoxic effect (Fig. 1a). Although cells treated with the essential oil of root from locality I displayed a slight increase in the number of DNA-damaged cells, no statistically significant differences were found between treated cells and the control (Fig. 1a). The results of treatment with three concentrations (25, 50 and 100 μg/mL) of the essential oil of aerial parts from both localities demonstrated the absence of genotoxicity, since the tested concentrations did not cause the induction of DNA damage (Fig. 1b). Similar results were shown after incubation with fruit essential oils at concentrations of 25, 50 and 100 μg/mL from both localities, i.e. fruit oils did not produce any statistically significant rise in DNA damage compared to the control (PBS), indicating a lack of genotoxicity (Fig. 1c). When the genotoxicity of root, aerial parts and fruit essential oils of the two different localities was compared, we found that there was no statistically significant difference (ANOVA, $p>0.05$).

Evaluation of antigenotoxic activity of essential oils

The antigenotoxic effect of the root, aerial parts and fruit essential oils from localities I and II against H_2O_2-induced DNA damage were examined using two experimental protocols, i.e. pre- and post-treatment. No statistically significant difference was observed in the number of DNA-damaged cells between cells treated with the oils before the treatment and the positive control treated only with H_2O_2. The root, aerial parts

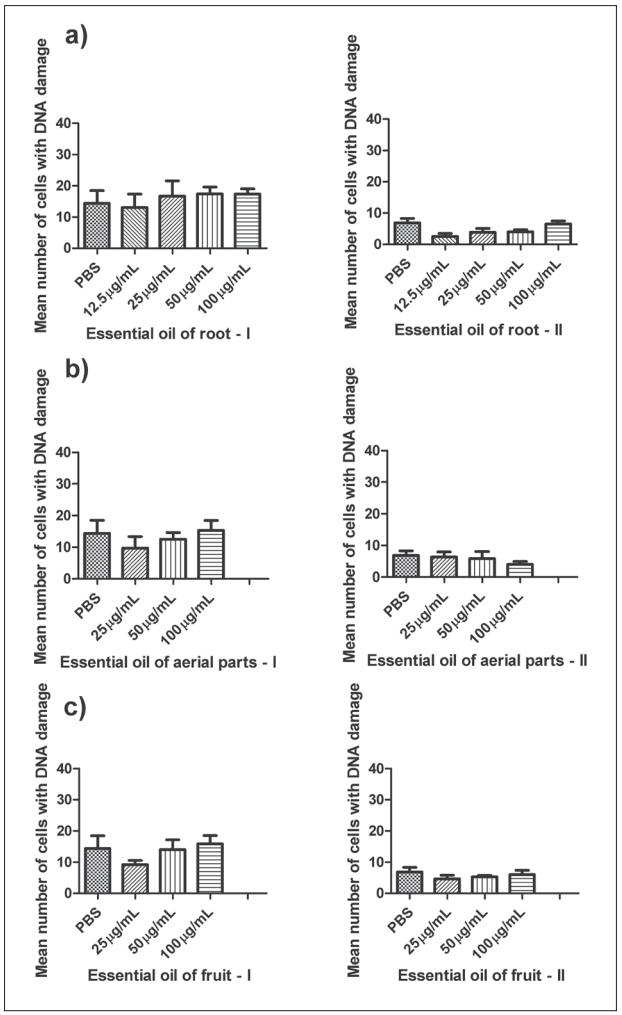

Fig. 1. Evaluation of the genotoxic potential of different concentrations of *Seseli rigidum* essential oils from root (A) aerial parts (B) and fruit (C) from locality I and II at the level of DNA damage. Graphs show the mean number of cells with damaged DNA±SEM.

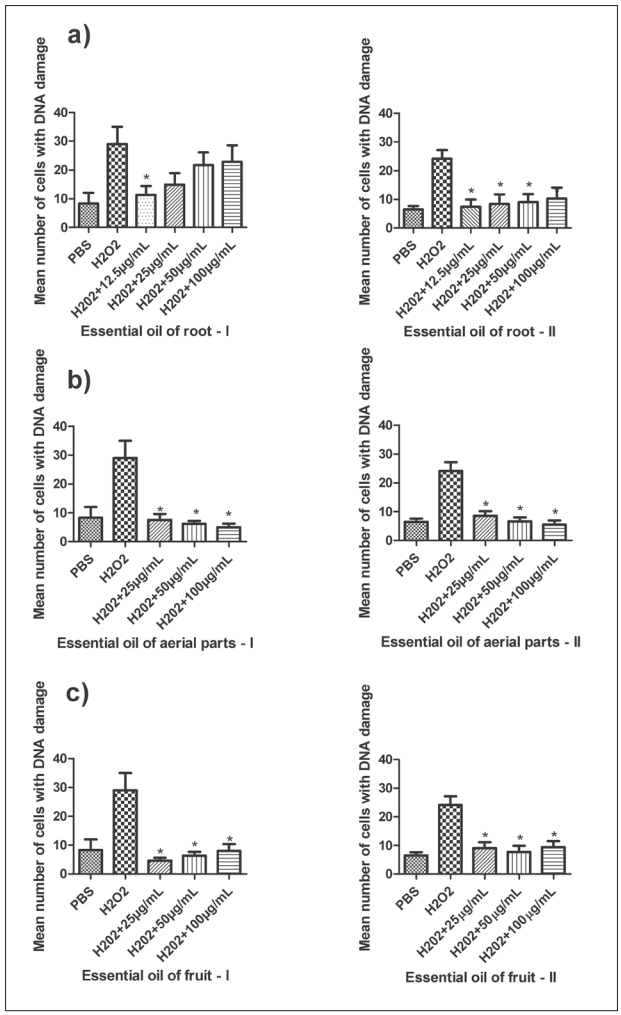

Fig. 2. Evaluation of the antigenotoxic potential of different concentrations of *Seseli rigidum* essential oils from root (A) aerial parts (B) and fruit (C) from locality I and II. Essential oils decreased the level of H_2O_2-induced DNA damage. Graphs show the mean number of cells with damaged DNA±SEM.; *vs H_2O_2, $P \leq 0.05$.

and fruit essential oils from the two localities applied in pretreatment (before the oxidant), did not prevent the induction of DNA damage by H_2O_2, indicating the absence of genoprotective activity of the examined oils (data not shown). The results of post-treatment conditions are given in Fig. 2. It was determined that all essential oils from the two localities applied post-treatment significantly decreased the number of cells displaying H_2O_2-induced DNA damage, pointing to strong antigenotoxic properties (Fig. 2). When the effect of root essential oil from locality I was examined, it was found that the number of cells with DNA damage was decreased compared to the control (H_2O_2) for all tested concentrations, although a significant difference ($p<0.05$) was obtained only for the lowest concentration of the root oil (12.5 μg/mL) (Fig. 2a). The results showed a concentration-dependent trend where a reduction in the concentrations of the root oil led to the decrease of H_2O_2-induced DNA damage. However, the antigenotoxic effect of the root oil from the locality II under the same conditions was more pronounced (Fig. 2a). Namely, incubation with

all tested concentrations of root oil from locality II displayed a statistically significant ($p<0.05$) decrease in H_2O_2-induced DNA damage compared to the positive control (45.4-67.1% of damage reduction). It should be noticed that the root oils from both localities showed the same concentration-dependent trend with smaller doses being more effective.The essential oils of aerial parts from both localities showed statistically significant ($p<0.05$) attenuation of H_2O_2-induced DNA damage (66.9-79.8%) for all tested concentrations (25-100 μg/mL) (Fig. 2b). Contrary to the effect of the root essential oil, the most pronounced DNA-damage reduction was achieved (79.8%) after incubation with the highest concentration of the oil of aerial parts (100 μg/mL). A significant antigenotoxic effect was also observed with the essential oil of fruit (55.6-81.4%) (Fig. 2c). The reduction in concentrations led to an increase in antigenotoxic effect and decrease in the number of DNA-damaged cells, in locality I.

Statistical analysis (ANOVA, $p>0.05$) showed that there were no significant differences between the antigenotoxic effects of *S. rigidum* root, aerial parts and fruit essential oils from the different localities.

DISCUSSION

Cell damage caused by oxidative stress can lead to a variety of degenerative changes linked with progression of a variety of diseases, including (Halliwell and Gutteridge, 1984). When the antioxidant defenses of a cell are overwhelmed by an excessive production of ROS, cellular macromolecules such as DNA undergo damage. The most common kind of damage due to an increase in ROS production is DNA oxidation (Azqueta et al., 2009). Since oxidative stress is recognized as an important factor in the etiology of many chronic diseases, the examination of dietary components with antioxidant activity is of increasing interest. Consequently, attention has been focused towards natural products as the source of novel antioxidants..

Significant protective effects and antioxidant activity of constituents of essential oils, like monoterpene hydrocarbons, have been described in several studies (Sinha et al., 2011; Lone et al., 2014; Woguem et al., 2013). In addition, α-pinene was considered as a natural antioxidant compound that may have beneficial health effects (Türkez and Aydin, 2013). Although the essential oils from aerial parts and fruit contained high amounts of monoterpenes (66.6-93.2%), especially α-pinene (21.1-57.4%) and sabinene (5.5-19.2%), their ferric reducing abilities (0-0.20 μmol Fe^{2+}/mg) and DPPH radical scavenging activities (SC_{50} 5.47-27.60 mg/mL) were low. On the other hand, such weak activity was comparable to the previously investigated DPPH scavenging activity of *S. rigidum* flower essential oil (24.5 μl/mL, which corresponds to about 24 mg/mL, depending on the density of the oil) (Stojković et al., 2009). The essential oil of root with its high polyacetylene falcarinol content showed higher reducing ability (0.38 μmol Fe^{2+}/mg locality I and 0.31 μmol Fe^{2+}/mg locality II) and DPPH radical scavenging activity (2.00 mg/mL locality I and 2.68 mg/mL locality II). Aliphatic C17-polyacetylenes of the falcarinol type occur in common food plants of the Apiaceae family and have demonstrated many activities such as antibacterial, anti-inflammatory, antiplatelet-aggregatory and cytotoxic effects (Christensen, 2011). Their bioactivity is probably based on the formation of stable carbocations, which act as very reactive alkylating agent towards biomolecules (Christensen and Brandt, 2006).

When the impact of essential oils of root, aerial parts and fruit of *S. rigidum* on nuclear DNA was investigated, no genotoxic effect was determined. However, the beneficial role of essential oils of *S. rigidum* in reduction of H_2O_2-induced DNA damage in whole blood cells was demonstrated. Essential oils of root and fruit from both localities displayed the same trend in antigenotoxic action. It was shown that a reduction in concentration led to higher antigenotoxic ability, indicating that the smaller concentrations were more effective.

The weak radical scavenging activity, but pronounced antigenotoxic concentration-dependent effect observed with essential oils of *Seseli rigidum* could be explained by the fact that the antioxidant activity of essential oils *in silico* does not predict their biologi-

cal effects in living cells. It was previously shown that some volatile terpenic and phenolic components of essential oils can function as antioxidant properties *in silico*, but after penetrating the cells, they are oxidized by ROS and converted into prooxidants. Conversion into a prooxidant may not occur if the antioxidant concentration is too weak and the switch from anti- to prooxidant reactions occurs in a very narrow range (Bakkali et al., 2008). Similar our results, Aydin et al. (2005) reported the low concentrations of thymol, carvacrol and γ-terpinene protected against oxidative DNA damage in lymphocytes, whereas high concentrations increased DNA breakage, implying that smaller concentrations were more effective against DNA damage.

Previous publications on the genoprotective activities of plant essential oils showed that some components of oils were able to protect cells against DNA damage induced by H_2O_2 (Slamenova et al., 2008; 2009). In our study, the essential oils of *Seseli rigidum* applied in pretreatment did not display protective ability against the damaging effect of H_2O_2. However, when the oils were incubated after exposure to H_2O_2 in the post-treatment regimen, a significant reduction in DNA damage was observed. Despite the evident differences in the composition of essential oils between the two localities, no statistically significant difference in their antigenotoxic activity was observed. The results of several studies showed that the antioxidant activity of whole plant essential oils is usually higher than that of a single biologically active compound. Also, the same authors suggested that the content of plant essential oils may vary according to the geographical origin of the same species, possibly affecting the antioxidant activity (Lado et al., 2004; Sacchetti et al., 2005). Our findings lead to the assumption that environmental conditions at the two different localities affected the composition of the oils but not their antigenotoxic abilities, since there were no significant dissimilarities in DNA damage reduction by the oils from roots, aerial parts and fruits of *S. rigidum* between different localities.

To elucidate the possible mechanism behind the antigenotoxic action of the essential oils, we performed two experiments, pretreatment and post-treatment, where the antigenotoxic activity of the oils could be displayed at a prevention or intervention level. In pretreatment conditions, the essential oils were added to cells 30 min before administration of the oxidant, allowing them to be active at the prevention level (Franke et al., 2005). Under pretreatment conditions oils may act by increasing the antioxidant capacity of the cells and free radical scavenging, making them more resistant to oxidative DNA damage (Cabarkapa et al., 2014). The results of pretreatment showed a very modest ability to decrease the number of DNA-damaged cells. The inability of *S. rigidum* oils to increase the protective capacity of the cells is in agreement with the obtained results of low antioxidant activity in the investigated oils. However, the post-treatment applied in our study led to significant attenuation of the deleterious effects of hydrogen peroxide at the intervention level. The antigenotoxic effect of essential oils seen in post-treatment could be assigned to the synergistic action of two independent mechanisms that possibly contributed in DNA-damage reduction: free radical scavenging and stimulation of DNA repair. Berić et al. (2008) demonstrated the ability of basil essential oil derivatives to remove already induced DNA damage by promoting mismatch DNA repair in *E. coli* K12 strains. Previous studies showed that 30 min are enough for DNA repair mechanisms to become active in cells (Chiaramonte et al., 2001). In our stydy, the cells were incubated with oil up to 30 min after administration of the oxidant, so the activation of DNA repair could significantly contribute to the overall antigenotoxic effect of the oils. Considering the above mentioned results of pre- and post-treatment, *S. rigidum* essential oils most likely have the ability to stimulate DNA repair mechanisms that significantly contribute to their antigenotoxic properties.

CONCLUSIONS

This study demonstrated that the tested concentrations of *S. rigidum* essential oils were not genotoxic under the described experimental conditions and were effective against the genotoxicity induced by hydrogen peroxide in whole blood cells. Although the essential

oils of all parts of *S. rigidum* from both localities exerted antigenotoxic activity, oils from the aerial parts and fruit showed the best antigenotoxic effect. Considering these findings, the *S. rigidum* oils could be proposed as a novel beneficial source of compounds with potential use in medical, cosmetic and pharmaceutical industries.

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