Comparison of chemical composition and biological activities of *Seseli rigidum* fruit essential oils from Serbia

**Abstract:** Plants from genus *Seseli*, have been widely used in European traditional medicine, exhibiting antibacterial, antifungal, insect repellent, emmenogogue, anti-flatulence, anti-inflammatory, antinoceptive, anti-tumor, anti-rheumatic activities and protective effect on human lymphocytes DNA. They usually grow on mountain rocky terrains. Part of their habitat on Vidlic Mountain, located in South-east Serbia, was struck with a large wildfire. *Seseli rigidum* fruit essential oils (from post fire and control areas) compositions were analyzed by GC and GC-MS, identifying monoterpenes α-pinene and sabinene as most abundant. Statistical tests showed a non-significant difference in chemical composition of these two oils, but a significant difference in comparison with the herb from a geographically different origin. Antimicrobial tests showed strong activities of the oils against tested bacteria, thus confirming its administration in various inflammation processes as a quite effective remedy. Applying DPPH· and ABTS·⁺ radical scavenging and total reducing Fe(III) to Fe(II) power assays, antioxidant characteristics of both studied essential oils were estimated as weak, though of close values. *Seseli rigidum* fruit essential oil was proven as a potent inhibitor of human and horse serum cholinesterase, recognizing its possible application as neural protective agent.

**Keywords:** antimicrobial, antioxidant, cholinesterase inhibition, *Seseli rigidum* essential oil

**1 Introduction**

*Seseli rigidum* belongs to the family Umbilliferae (Apiaceae). It is a perennial plant with very developed root; leaves are coarse, grayish-green and double plumose; inflorescences are large with white flowers, blooming in July and August. Fruits are cylindrical with very prominent ribs. This is an endemic plant, growing mainly in Serbia, Bosnia and Herzegovina, Montenegro, Macedonia, and Turkey on stony and rocky terrains, on limestone or silicate pads. Plant organs are of characteristic anatomy, containing oil reservoirs which are distributed in different parts of the plant. The amount and composition of the essential oils mainly depends on the organ and growth stage of the plant [1].

Numerous species of genus *Seseli* have been widely used in traditional medicine because of their antibacterial, antifungal, insect repellent activities [2-7], as well as an emmenogogue, anti-flatulence [8], anti-inflammatory, antinoceptive [9], anti-tumor [10], anti-rheumatic activities [11] and protective effect on human lymphocytes DNA [12]. Genus *Seseli* is represented in Serbia by ten species [13], including *Seseli rigidum* Waldst. & Kit., famous in ethno medicine as an herb, with magical healing properties [14]. The presence of herb *Seseli rigidum* was recorded at several sites in Serbia, mostly on higher altitudes [1,15-17].

Wildfire on the mountain Vidlic (South-east Serbia), occurred in the summer of 2007 and swept through a large area of open habitats to dry grasslands and rocky terrains to an area of about 1000-1500 ha [18]. Impact of wildfire on the open habitat and vegetation recovery has been studied recently in South African grasslands [19,20] and in Mediterranean ecosystems [21-24], primarily by monitoring impact of fire on seed plants. Plants that have been survived wildfire in the open grassy areas in the form of seeds are mostly annually, but some perennial as well, mainly due to production of large numbers of tiny seeds or in the form of underground organs [25-27].

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Qualitative composition of the fruit essential oil of *S. rigidum* was reported [1], but only 10 monoterpenes were registered and 8 identified. The most abundant was α-pinene, followed by sabine and ocimene, while the others were registered in traces and among sesquiterpenes caryophyllene and elemene were recorded. Although α-pinene is a major component of flower *S. rigidum* essential oil, there were significant differences in the presence and representation of accompanying components [16]. In the aerial essential oil of *S. rigidum* from Western Serbia, α-pinene was reported as predominant compound (57.4%), followed by limonene, camphene and sabine. For the fruit essential oil of the same plant, dominant compounds were α-pinene (23.3%), β-phellandrene and sabinene. Surprisingly, the root essential oil was composed almost of the falcarinol (88.8%) [17].

Antimicrobial and antioxidant activities of plant natural products have become an unavoidable tool in understanding and estimating their validity as remedies. Because of evidence of pro oxidant effects on the cellular level, essential oils could play a very important role through exhibiting cytotoxic but usually nongenotoxic effects [28]. Antimicrobial activity was tested for essential oils from post fire and control areas, against the most common bacterial and fungal strains. Among plant secondary metabolites, polyphenols are known to have a high capacity to scavenge free radicals due to the hydrogen and electron donating abilities of their hydroxyl groups and in that way are considered responsible for antioxidant action. High content of non-phenolic compounds (monoterpenes and sesquiterpenes) in most essential oils might be related to their weak antioxidant activity [29]. For fruit essential oils, originating from post fire and control plant samples, antioxidant characteristics were performed in order to compare them to each other, and also to data for the same oil from different geographical location.

Cholinesterase inhibition is considered to play important role in prevention and control of the Alzheimer’s disease, an increasing problem of modern man-kind. Since some terpenes, especially α-pinene, significantly inhibit acetylcholinesterase [30], we decided to examine *S. rigidum* fruit essential oil impact on cholinesterase activity, considering α-pinene as its dominant compound, disregarding the locality in which plant material was collected.

With the exception of chemical composition, antimicrobial, antioxidant activities and interaction with cholinesterase essential oils from post fire and control areas, were also compared with published parameters of *S. rigidum* essential oil from a geographically diverse origin [17]. This was in order to infer if the wildfire or completely different environmental conditions are crucial for the studied essential oils characteristics.

To the best of our knowledge this is the first report of post fire *S. rigidum* fruit essential oil composition. Its biological activities, such as antimicrobial action against Gram positive bacteria *Bacillus cereus*, and fungal strain *Candida albicans*, *Aspergillus niger*, may relate to its traditional use. Antioxidant characteristics estimated by total reducing power and ABTS+ assay and behavior as cholinesterase inhibitors, are also reported for the first time.

## 2 Experimental procedure

### 2.1 Plant

*Seseli rigidum* Waldst. & Kit. (Apiaceae) fruits were collected on the rocky post fire terrains and areas nearby not affected by fire, on Vidlic Mountain in South-eastern Serbia in July 2009. A voucher specimen (16447) was deposited in the Herbarium of the Botanic Garden "Jevremovac", Faculty of Biology, University of Belgrade (BEOU).

### 2.2 Tested material

Essential oils were obtained by hydro distillation of fruits in a Clevenger type glass apparatus (control area: yield=0.33%, d=0.350 g mL⁻¹; post fire area: yield=0.34%, d=0.351 g mL⁻¹).

### 2.3 Analysis of essential oil

The volatile oil was analyzed by GC/MS and GC analyses (3 repetitions of each sample) using a Hewlett-Packard 6890 N gas chromatograph equipped with a fused silica capillary column HP-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company.

The injector and interface operated at 250 and 300°C, respectively. Oven temperature was raised from 70 to 290°C at a heating rate of 5°C min⁻¹ and then isothermally held for 10 min. The carrier gas was helium with a flow of 1.0 mL min⁻¹. The samples, 1 μL of the oil solutions in diethyl ether (1:100), were injected in a pulsed split mode.
(the flow rate was 1.5 mL min\(^{-1}\) for the first 0.5 min and then set to 1.0 mL min\(^{-1}\) throughout the remainder of the analysis; split ratio 40:1). MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35-500, scan time 0.32 s.

Oil constituents were identified by comparison of their linear retention indices (relative to C\(_6\)–C\(_{33}\) alkanes [30] on the HP-5MS column) with literature values [31] and their MS with those of authentic standards, as well as those from Wiley 6, NIST02, Mass Finder 2.3 [32] and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils by the application of the AMDIS software (Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, DTRA/NIST, 2002). Some components were identified by co-injection with an authentic sample [33]. GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition of the oil was computed from the GC peak areas without any corrections.

### 2.4 Antimicrobial assay

The antimicrobial activity of essential oils (the minimum inhibitory concentration- MIC and minimum bactericidal/fungicidal concentration- MBC/MFC) were investigated using micro well-dilution method against laboratory control strains obtained from the American Type Culture Collection [34] and according to the National Committee for Clinical Laboratory Standards (NCCLS 2003). The inocula of the bacterial strains were prepared from the overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity (corresponding to \(10^7-10^8\) CFU mL\(^{-1}\), depending on genera- consensus standard by the NCCLS). The density of the fungal spores was determined by entering them in sterile physiological solution and their subsequent counting in a Thoma chamber under the microscope. Serial doubling dilutions of the tested samples were prepared in Mueller-Hinton broth (for bacteria) and in Sabouraud Dextrose broth (for yeasts and fungal strains) in a 96/well microtiter plate over the range of 0.2-50.00 μL mL\(^{-1}\) in inoculated nutrient broth. The final volume was 100 μL, the final concentration was \(10^6\) CFU mL\(^{-1}\) and \(10^5\) fungal spores mL\(^{-1}\) in each well. The plate was incubated at 37°C for 24 h (bacteria strains) and at 25°C for 48 h (yeast and filamentous fungal strains). All experiments were performed in triplicate. Two controls were included- medium with ethanol (negative control) and medium with tetracycline and nystatin (positive control). Microbial growth was determined by adding 20 μL of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution [36]. Minimal inhibitory concentration (MIC) was defined as the lowest concentration of the samples inhibiting visible growth (red colored pellet on the bottom of the wells after the addition of TTC), while minimal bactericidal/fungicidal concentration (MBC/MFC) was defined as the lowest samples concentration killing 99.9% of microbial cells. To determine MBC/MFC, the broth was taken from each well without visible growth and inoculated in Mueller-Hinton agar (MHA) for 24 h at 37°C for bacteria and in Sabouraud Dextrose Agar (SDA) for 48 h at 25°C for fungal strains and yeasts.

### 2.5 Antioxidant assay

Antioxidant properties were estimated by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) and Fe(III) to Fe(II) reducing antioxidant power assay [30].

#### 2.5.1 DPPH• radical scavenging assay

The DPPH•-assay was performed as described [30]. Essential oils (10 μL) were mixed with 90 μmol L\(^{-1}\) DPPH in methanol (1.0 mL), and these solutions were diluted up to 4.0 mL. After shaking mixture vigorously, they were stored in darkness for 60 min at room temperature and the absorbances were measured at 515 nm.

Radical scavenging activity of the samples was expressed as \(SC_{50}\) (concentration of antioxidant, which produces scavenging of 50% DPPH radicals). In order to enable wider comparability with literature data, besides BHT (2,6-bis(1,1-dimethylethyl)-4-methylphenol) applied in the numerous experiments as referent standard, the usual standard antioxidant compounds such as trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ascorbic acid, rutin and gallic acid were subjected to the same procedure.

#### 2.5.2 ABTS** radical cation assay

The antioxidant capacity of the samples was expressed as \(EC_{50}\) (concentration of antioxidant, which produces scavenging of 50% ABTS radicles). In order to enable wider comparability with literature data, besides BHT (2,6-bis(1,1-dimethylethyl)-4-methylphenol) applied in the numerous experiments as referent standard, the usual standard antioxidant compounds such as trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ascorbic acid, rutin and gallic acid were subjected to the same procedure.
Generation of radicals before the antioxidants are added prevents interference of compounds, which affects radical formation. This modification makes the assay less susceptible to artifacts and prevents overestimation of antioxidant capacity [37]. When stable absorbance is obtained the antioxidant sample is added to the reaction medium, and the antioxidant activity is measured in terms of decolorization.

Fifty microliters of samples were mixed with 1.9 mL of diluted ABTS solution. The mixture was allowed to stand for 6 min at room temperature and the absorbance was immediately recorded at 734 nm. Trolox solution (final concentration 0-15 µM) was used as a reference standard. The results were expressed as trolox equivalents (µg mL⁻¹) oil.

2.5.3 Total reducing power assay Fe(III) to Fe(II)

The reducing power of the oils were determined as described [38] and expressed in relation to the reducing power of ascorbic acid as a positive control (Ascorbate Equivalent Antioxidant Capacity). The sample (10 µL) was mixed with K₃[Fe(CN)₆] (1 mL, 1%) and NaH₂PO₄·Na₂HPO₄ buffer (1 mL, 0.2 mol L⁻¹, pH 6.6). These mixtures were incubated at 50°C for 30 min, then trichloroacetic acid (1 mL, 10%) was added and mixtures were centrifuged at 3000 rpm for 10 min. Finally, the supernatants (1 mL) were mixed with distilled water (1 mL) and FeCl₃ (0.2 mL, 0.1%) and absorbencies were measured at 700 nm. The reducing power of the samples was expressed as µg of ascorbic acid per mg of essential oil or trolox/rutin/gallic acid.

2.6 Cholinesterase inhibition properties

2.6.1 Human cholinesterase source for the assay

Ten healthy volunteers (18-65 years old from both sexes) from the Pirot General Hospital, donated blood with written consent. According to the questionnaire, nobody had serious medical disorders, nor had been abusing drugs, cigarettes, or alcohol. At least one month before the blood donation, none of them had been administrating any medication. From each participant, 5 mL blood sample were collected in a Vacutainer tube, centrifuged at 3000 rpm for 10 min and the serum supernatant was pooled and used as the source of the enzyme for the assay.

2.6.2 Procedure

Serum cholinesterase (disregarding its origin) catalyzes the hydrolysis of butyrylthiocholine to thiocholine, which reacts with chromogen dithio-nitro benzoic acid (DTNB). The reaction rate is determined from the rate of 5-thio-2-nitro benzoic acid formation, measured at 405 nm, in six cycles of 28 s. Analyzed solutions (samples of essential oils, α-pinene or reference standard solution) (10 µL) were mixed with 10 µL of the pooled serum (diluted with the phosphate buffer in ratio 1:9, v/v), and the phosphate buffer solution (160 µL). These were reinsulated for 10 minutes (at 310 K) when a DTNB solution (10 µL) was added. After 60 sec, substrate solution (BuTC, 10 µL) was added [39]. As the reference standard, a solution of neostigmine bromide was used in the concentration of 200 µg mL⁻¹.

2.6.3 Horse serum cholinesterase assay

Horse serum cholinesterase, EC 3.1.1.8, (Sigma Aldrich, St Louis, MO, USA) was used as an enzyme source, according to the above procedure.

2.7 Statistical analysis

Statistical analysis of the chemical composition data for the two studied essential oils (from post fire and control areas) and one previously published [17], was carried out using Mathworks MATLAB software. Hierarchical cluster analysis (HCA) is a multivariate technique, aimed to classify the objects of the system into categories or clusters based on their similarities [40]. Principal component analysis (PCA), often reported in literature [41,42], also enables grouping of the samples, by means of Ward’s method using Euclidean distances as a measure of similarity. Here is the PCA analysis was applied to reduce the number of observed variables (constituents of essential oils) to a smaller number of principal components that account for most of the variance of the observed variables.

3 Results and discussion

Comparative overview of the studied essential oils chemical composition, and data reported by Marcetic et al. [17] is presented in Table 1.
Antimicrobial screening of fruit essential oils of *S. rigidum* (from post fire and control areas), to six microbial strains that cause most frequently food spoilage and gastrointestinal illness in humans (Table 2), were performed and the results are presented in Table 2.

Antioxidant characteristics of the *S. rigidum* essential oils originated from post fire and control areas were assessed employing three different methods (Table 3).

A total of 35 components were identified representing 94.4% of *S. rigidum* fruit oil from control area (Table 1). Two major groups of identified compounds were monoterpene and sesquiterpene hydrocarbons (83.9% and 10.5%, respectively). The most abundant compound in the oil was monoterpene α-pinene (37.8%), followed by sabinene (13.5%).

In the fruit essential oil from the post fire area, 36 components were identified representing 95.9% of the oil (Table 1). Two major groups of identified compounds were monoterpene and sesquiterpene hydrocarbons (84.3% and 11.6%, respectively), spotting α-pinene (36.2%) as dominant component, followed by sabinene (14.2%). The examined oil contained thuj-2,4(10)-diene (0.1%), while in the oil from area not affected by fire was not detected at all.

The fruit essential oil composition of the analyzed *S. rigidum* species, were compared to the previously published data concerning fruit essential oil of the same plant, collected at different locality [17], in order to explore and clarify potential influence of genetic, geographical and environmental factors, on the chemical composition of *S. rigidum* fruit essential oil.

Classification and comparison of essential oils based on their chemical composition can only be objectively rated by utilization of appropriate chemometric methods. Interpretation and discussion of results, presented by tables or/and graphics can be extremely subjective. Comparison of essential oils based on their chemical composition was made by multivariate statistical analyses. The principal component analysis (PCA) and hierarchical cluster analysis (HCA) separated studied oils in two groups. The statistical approach showed no significant difference between samples from post fire and control area, giving us arguments to consider the impact of wild fire negligible on chemical composition of *S. rigidum* essential oil. On the other hand, a divergence between our study and previously published results could be explained by diversities among plants growing in different geographical areas.

Score and loading plots of studied samples, with respect to their chemical composition, pointed similarity between samples from the same area (Fig. 1A) irrespective of wildfire, and significant differences between these essential oils and the sample from the different region. The HCA based on the Euclidean distance indicated two groups of samples (A and B), which were identified by essential oil chemical composition with a dissimilarity ≥19.7 (Fig. 1B). With a dissimilarity ≥2.7 group A was divided in two sub-groups (A₁ - *S. rigidum* grown on control area and A₂ - *S. rigidum* grown at same geographical location on post wildfire area). Group B was constituted by the chemical composition of *S. rigidum* fruit essential oil, researched by Marcetic et al. [17].

Score and loading plots of a matrix constituted by the chemical compound classes (monoterpane hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes) also confirmed greater chemical similarity between *S. rigidum* grown on identical geographical location (Fig. 2A). PC1 horizontal axis explained 94.67% while PC2 vertical axis explained 5.33% of the total variance.

The HCA based on the Euclidean distance indicated two groups of species (C and D), which were identified by their essential oil compound classes with a dissimilarity ≥12.5 (Fig. 2B). With a dissimilarity ≥3.6, the group C, with a considerable amount of monoterpene hydrocarbons and oxygenated monoterpenes was divided in the two sub-groups C₁ and C₂. With the value of the PC2 axis in mind, the difference between sub-groups is negligible, in order to compare the contents of dominant chemical classes. Slightly higher values of ratio monoterpene hydrocarbons/oxygenated monoterpenes in group C₂ could be explained by deprivation of oxygen in soil as a consequence of wildfire. *Seseli rigidum* researched by Marcetic et al. [17] formed a separate chemical D group in PCA and HCA analysis with considerable amount of sesquiterpenes (Figs. 2A-2B).

Many plants produce essential oils, known to possess biological activity against prokaryotic and eukaryotic organisms [43]. The oils were active against all strains tested, ranging from 0.03 to 6.50 µL mL⁻¹ (0.01 to 2.28 mg mL⁻¹). Manifested activity can be considered as a good in comparison to the effects of referent antibiotics (tetracycline was active against bacterial strains in the range of 0.7-4.0 mg mL⁻¹ while nystatin expresses activity against fungal strains in the range of 8.0-12.0 mg mL⁻¹). It was also observed that the inhibitory and microbiocidal oil concentrations were the same for *S. aureus* (0.01 mg mL⁻¹), while for the rest of the investigated strains inhibitory concentrations were higher (0.04 mg mL⁻¹) as well as microbiocidal concentrations, ranging from 0.07-1.13 mg mL⁻¹. The best activity was demonstrated against bacterial strain *Staphylococcus aureus* ATCC 6538 (MIC=MBC=0.03 µL mL⁻¹, 0.01 mg mL⁻¹), while the weakest effect was on germination of fungal spores of *Aspergillus niger* ATCC 10231 (MIC=MBC=6.25-6.50 µL mL⁻¹,
2.19-2.28 mg mL\(^{-1}\)). In Gram negative bacteria, concentrations of oil needed to provoke microbicidal effect, were significantly higher in relation to the inhibitory concentrations, which could be explained by complex structure of their cell walls, and in the strain of \textit{P. aeruginosa}, by additional hydrophilic coating that prevents entry of the oil into the cells interior. There was no significant difference between activities of the fruit essential oil isolated from plants grown on area not affected by fire and post fire areas, because the proportion of the dominant components in the oil from both sites is similar, and the components with larger share are: \(\alpha\)-pinene (36.2-37.8%), sabinene (13.5-14.2%), \(\beta\)-phellandrene (5.1-5.2%) and germacrene B (2.8-3.2%). It is alreday known that \textit{S. rigidum} flower essential oil, containing high amount of \(\alpha\)-pinene (48.5%) posses good antimicrobial activity [16]. However, \(\alpha\)-pinene was previously identified as antimicrobial agent [44]. The investigated fruit essential oils of \textit{S. rigidum} didn’t contain compound falcarinol, which was detected in the fruit oil (3.0%) and oil from the roots (88.8%) of the same plant species collected in the Western Serbia [17]. This compound is well known antimicrobial agent [45-47], considered as responsible for the high activity of the root essential oil to Gram

| No. | Component | I, %  | II, %  | III, % |
|-----|-----------|------|-------|--------|
| 1   | Monoterpene hydrocarbons | 73.9 | 71.6  | 68.7   |
| 2   | \(\alpha\)-thujene | 0.6  | 0.4   | –      |
| 3   | \(\alpha\)-pinene | 37.8 | 36.2  | 23.3   |
| 4   | camphene | 2.8  | 1.9   | 2.3    |
| 5   | thuja-2,4(10)-diene | –    | 0.1   | –      |
| 6   | \(\beta\)-pinene | 3.3  | 2.8   | 2.1    |
| 7   | \(\beta\)-myrcene | 1.3  | 1.4   | 3.4    |
| 8   | \(\alpha\)-phellandrene | 1    | 1.2   | 3.9    |
| 9   | \(\alpha\)-terpinene | 0.8  | 0.6   | –      |
| 10  | \(p\)-cymene | 1.3  | 1.3   | 1.2    |
| 11  | limonene | 3.4  | 2.5   | –      |
| 12  | \(\beta\)-phellandrene | 5.1  | 5.2   | 17.4   |
| 13  | \(\gamma\)-terpinene | 2.8  | 3.5   | 2.2    |
| 14  | \(p\)-mentha-2,4(8)-diene | 0.2  | 0.3   | –      |
| 15  | \(\text{Oxygenated monoterpenes}\) | 10.0 | 12.7  | 2.9    |
| 16  | \(\text{cis-sabinene hydrate}\) | 0.4  | 0.7   | –      |
| 17  | \(\text{trans-sabinene hydrate}\) | 0.2  | 0.4   | –      |
| 18  | \(\alpha\)-campholenal | 1.2  | 1.5   | –      |
| 19  | \(\text{trans-pinocarveol}\) | 1.1  | 1.3   | –      |
| 20  | \(\text{trans-verbolen}\) | 1.4  | 1.5   | –      |
| 21  | pinocarone | 0.8  | 1.1   | –      |
| 22  | \(\text{cis-pinocamphone}\) | 0.3  | 0.4   | –      |
| 23  | terpinen-4-ol | 2.5  | 3.2   | 0.8    |
| 24  | myrtenal | 0.7  | 0.9   | –      |
| 25  | \(\text{Oxygenated sesquiterpenes}\) | 3.6  | 4.1   | 6.5    |
| 26  | \(\text{caryophyllene oxide}\) | 0.9  | 1     | 0.6    |
| 27  | carotol | 0.6  | 0.4   | 2.3    |
| 28  | \(\beta\)-oplopenone | 0.6  | 0.6   | 0.5    |
| 29  | muurola-4,10(14)-dien-1-\(\beta\)-ol | 2.1  | 2.7   | 3.1    |
| 30  | falcarinol | 2.1  | 2.7   | 3.1    |
| 31  | \(\text{Others}\) | –    | –     | 3.0    |

Table 1: Essential oil composition of \textit{S. rigidum} collected in: control area (I); post fire area (II); another geographical location (III- Marcetic et al., 2012).
positive strains, especially against methicillin resistant S. aureus (in the range 6.25-50.00 µg mL⁻¹) [17]. Such a good activity of the tested oils could be explained by high content and favorable ratio of two components: α-pinene (37.8%) and sabinene (13.5%). Better activity of the tested oils, compared to oil from the site in Brdjanska gorge in west Serbia [17] may be contributed to the presence of larger number of components present in low amounts, provoking a possible synergistic effect. In addition, the influence of p-cymene and γ-terpinene, known by tendency to incorporate in cytoplasmatic membranes of microbes, may be manifested, enabling gateway for the other active compounds [48].

The free radical scavenging capacity was estimated by applying DPPH assay through concentrations of oils which scavenged 50% of DPPH radical (SC⁵₀), and were 16.93±0.40 mg mL⁻¹ (48.36±1.13 µL mL⁻¹) and 18.75±0.39 mg mL⁻¹ (53.42±1.12 µL mL⁻¹) for essential oil from control and post fire areas, respectively. Comparing obtained data to already published for flower essential

| Table 2: Antimicrobial activities of S. rigidum fruit essential oils. |
| --- |
| **Microbial strain** | **Essential oil (not affected by fire) (MIC/MBC in µL mL⁻¹)** | **Essential oil (not affected by fire) (MIC/MBC in mg mL⁻¹)** | **Essential oil (post fire) (MIC/MBC in µL mL⁻¹)** | **Essential oil (post fire) (MIC/MBC in mg mL⁻¹)** | **Antibiotic (MIC/MBC in µg mL⁻¹)** |
| **Gram (-) bacteria** | | | | | |
| *Escherichia coli* (ATCC 25922) | 0.12/1.60 | 0.04/0.56 | 0.10/1.56 | 0.04/0.55 | 2.0/4.0 |
| *Pseudomonas aeruginosa* (ATCC 27853) | 0.12/3.24 | 0.04/1.13 | 0.10/3.12 | 0.04/1.10 | 2.0/4.0 |
| **Gram (+) bacteria** | | | | | |
| *Staphylococcus aureus* (ATCC 6538) | 0.03/0.03 | 0.01/0.01 | 0.03/0.03 | 0.01/0.01 | 0.7/1.40 |
| *Bacillus cereus* (ATCC 10876) | 0.12/0.24 | 0.04/0.08 | 0.10/0.20 | 0.04/0.07 | 1.80/1.80 |
| **Fungal strain** | | | | | |
| *Candida albicans* (ATCC 16404) | 3.24/3.24 | 1.13/1.13 | 3.12/3.12 | 1.10/1.10 | 8.00/8.00 |
| *Aspergillus niger* (ATCC 10231) | 6.50/6.50 | 2.28/2.28 | 6.25/6.25 | 2.19/2.19 | 12.0/12.0 |

| Table 3: Antioxidant activity of fruit essential oil of S. rigidum from non affected by fire and post fire area (DPPH, ABTS⁺ and FRAP). |
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| **Assay** | **Sample** | **Essential oil (not affected by fire)** | **Essential oil (post fire)** | **BHT** | **Trolox** | **Ascorbic acid** | **Rutin** | **Gallic acid** |
| | | | | | | | | |
| **DPPH** (SC⁵₀) (mg mL⁻¹) | 16.93±0.40 | 18.75±0.39 | 16.51±1.08 | 1.50±0.09 | 0.56±0.02 | 1.43±0.11 | 0.29±0.01 |
| **ABTS⁺** (µg mL⁻¹ trolox equivalents) | 0.69±0.02 | 0.66±0.01 | 3.45±0.04 | / | 2.78±0.03 | 3.34±0.07 | 3.02±0.06 |
| **FRAP** (µg ascorbic acid per mg essential oil or substance) | 0.24±0.01 | 0.30±0.02 | 7.30±0.02 | 5.04±0.04 | / | 9.71±0.27 | 7.58±0.15 |
oil (24.5 μL mL⁻¹) [16], at first sight it could be observed it was about two times weaker than the activity of tested fruit essential oils. Such a huge discordance may be contributed to the different biological and geographical origin of the samples. However, comparison of SC₅₀ values registered for the usual standard substances in both experiments, led us to the conclusion of possible modified experimental procedures applied in compared experiments. Since the publication of Stojkovic et al. [16], doesn’t contain description of DPPH assay applied, it is the only logical explanation. When compared, studied antioxidant activities expressed in the same units as referent substances, consideration of them as weak antioxidant becomes more obvious - over thousand more of each essential oil is required to produce 50% scavenging action in comparison to 16.51 μg of BHT, 1.50 μg of trolox, 0.56 μg of ascorbic acid, 0.43 μg of rutin and 0.29 μg of gallic acid which causes the same effect.

In order to confirm radical scavenging characteristics of the studied essential oils, and bearing in mind the slight disagreement with the published results, another assay that includes ABTS⁻ radical was performed, and values of 0.66±0.01 μg mL⁻¹ and 0.69±0.02 μg mL⁻¹ trolox equivalents was obtained for the sample from post fire and areas not affected by fire, respectively. Both values differ slightly, which is in accordance with data obtained by DPPH assay, but they still remain low, enabling the final estimation of their antiradical characteristics as weak.

Numerous publications dealing with antioxidant potentials of the essential oils frequently refer to terms such as additivity, synergism and antagonism. Among plant metabolites, phenols have been confirmed as...
compounds with strong antioxidant activity [49], but unfortunately, they are not located in the essential oils. The group of compounds mainly responsible for the antioxidant potential of the plant essential oils, are in particular, oxygenated monoterpenes [50], while monoterpenoid hydrocarbons possess lower potentials as antioxidants [51].

Total reducing power of *S. rigidum* fruit essential oils was estimated by Fe(III) to Fe(II) reducing power assay, expressed as ascorbic acid equivalents and compared to the four most usually applied antioxidant standards such as BHT, trolox, rutin and gallic acid. The values of 0.24±0.01 and 0.30±0.02 µg mL−1 for samples originated from area not affected by fire and post fire areas, respectively, are very similar, indicating no significant influence of wildfire on total reducing power properties. Such a result could be linked to the oxygenated monoterpenes share in the studied essential oils, observing correspondence of oxygenated monoterpenes share with total reducing power. In comparison to the set of standards, applied in the previous assays, total reducing power of the tested essential oils could be estimated as low.

Considering *S. rigidum* essential oil from post fire area, inhibition of pooled human and horse serum cholinesterase was significant (34.9±0.04% and 41.78±0.06% respectively) referring to neostigmine bromide as standard compound (78.56±0.77% and 98.10±0.83% respectively). The essential oil isolated from the plant grown in the area not affected by fire, exhibited very similar activities against pooled human and horse serum cholinesterase (33.45±0.02% and 40.23±0.04% respectively). There is no significant difference in action of both studied essential oils to inhibition, both essential oils acted as strong inhibitors, even higher than effect of their dominant constituent α-pinene, leading to the conclusion that the other constituents of the essential oil manifested their action toward cholinesterase, without looking into whether it was additive or synergistic.

Considering human and horse serum cholinesterase inhibition, both essential oils acted as strong inhibitors, even higher than effect of their dominant constituent α-pinene, leading to the conclusion that the other constituents of the essential oil manifested their action toward cholinesterase, without looking into whether it was additive or synergistic.

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