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Antioxidant activity of oregano essential oil (*Origanum vulgare* L.)

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***Abstract*:**

Stanojević, Lj.P., Stanojević, J.S., Cvetković, D.J., Ilić, D.P.: *Antioxidant activity of oregano essential oil (Origanum vulgare L.). Biologica Nyssana, 7 (2), December 2016: 131-139.*

Essential oil obtained from oregano (*Origanum vulgare* L.) by Clevenger-type hydrodistillation and hydromodulus 1:10 m/v during 180 minutes, has been investigated in this work. Qualitative and quantitative composition of the oil was determined by GC-MS and GC-FID spectrometry. Antioxidant activity of the obtained oil was examined spectrophotometrically by DPPH test (after 20, 30, 45 and 60 minutes of incubation) and TBA-MDA assay. The yield of essential oil was 4.1 mL/100 g of plant material. Seven components were identified: α-thujene, myrcene, α-terpinene, o-cymene, γ-terpinene, thymol and carvacrol. The major components were thymol (45%) and carvacrol (37.4%). Oil incubated for 60 minutes has shown the best antioxidant activity according to DPPH test. The concentrations of essential oil, required for neutralization of 50% of initial DPPH radical concentration (EC_{50}), were 0.761, 0.590, 0.360 and 0.326 mg/mL, after 20, 30, 45 and 60 minutes of incubation, respectively. Lipid peroxidation inhibition of 92.3% was achieved by 1.35 mg/mL essential oil concentration. The results obtained indicate that oregano essential oil is a good source of natural antioxidants with potential application in food and pharmaceutical industries, as a safer alternative to the synthetic antioxidants.

Key words: *Origanum vulgare* L., essential oil, antioxidant activity, GC-MS analysis

***Apstrakt*:**

Stanojević, Lj.P., Stanojević, J.S., Cvetković, D.J., Ilić, D.P.: *Antioksidativna aktivnost etarskog ulja vranilove trave (Origanum vulgare L.). Biologica Nyssana, 7 (2), December 2016: 131-139.*

U ovom radu je ispitivano etarsko ulje vranilove trave (*Origanum vulgare* L.) dobijeno Clevenger hidrodestilacijom pri hidromodulu 1:10 m/v, u toku 180 minuta. Kvalitativni i kvantitativni sastav dobijenog etarskog ulja određen je GC-MS i GC-FID spektrometrijom. Antioksidativna aktivnost ulja je određena spektrofotometrijski, DPPH testom (posle 20,30, 45 i 60 minuta inkubacije) i TBA-MDA testom. Prinos etarskog ulja bio je 4.1 mL/100 g biljnog materijala. Identifikovano je sedam komponenti: α-tujen, mircen, α-terpinen, o-cimen, γ-terpinen, timol i karvakrol. Kao glavne komponente identifikovane su timol (45%) i karvakrol (37,4%). Najbolju DPPH-antioksidativnu aktivnost pokazalo je ulje nakon 60 min inkubacije. Koncentracije etarskog ulja neophodne za neutralizaciju 50% od početne koncentracije DPPH radikala (EC_{50} vrednosti) bile su 0,761; 0,590; 0,360 i 0,326 mg/mL, nakon 20, 30, 45 i 60 min inkubacije, respektivno. Inhibicija lipidne peroksidacije od 92,3% je postignuta sa 1,35 mg/mL eterskog ulja. Dobijeni rezultati sugerišu da dobijeno etarsko ulje vranilove trave predstavlja dobar izvor prirodnih antioksidanasa sa potencijalnom primenom u prehrambenoj i farmaceutskoj industriji kao bezbednija alternartiva sintetskim antioksidansima.

Ključne reči: *Origanum vulgare* L., etarsko ulje, antioksidativna aktivnost, GC-MS analiza

Introduction

The oxidation process is one of the major causes of food spoilage, which results in rancidity and deterioration of the nutritional quality, color, flavor, texture, and safety of foods (Betaieb et al., 2010). A significant number of herbs is used as natural preservatives in food industry. Besides being used to achieve the proper flavor and to intensify the flavors some spices and herbs exhibit antioxidant effects which is of great importance for food industry (Stanković & Stanojević, 2014; Tongnuanchan et al., 2014).

Plants rich in antioxidant compounds are present in food industry (Betaieb et al., 2010). There are evident requirements for increasing application of natural antioxidants obtained from plant material. Undesirable side effects of synthetic antioxidants, such as butyl hydroxy anisole (BHA) and butylated hydroxytoluene (BHT), which are associated with their toxic and carcinogenic effects (Maestri et al., 2006), are the reason of such requirements.

Essential oils are very heterogeneous group of complex mixtures of secondary plant metabolites. Composition of essential oil may be different between different species or varieties, related to different cultivation, origin, vegetative stage and growing seasons of the plant (Vazirian et al., 2015). Beside aroma, odor and fragrance of many of the oils, some of them have also been confirmed to possess antioxidant activities (Betaieb et al., 2010; Vazirian et al., 2015). Essential oils are also widely used in perfumes, cosmetics, aromatherapy and nutrition (Burt, 2004; Bakkali et al., 2008).

Earlier studies have been shown that, among the herbs and spices which are extensively studied, the plants from the *Lamiaceae* (*Labiatae*) family possess a significant antioxidant activity (Tsimidou & Boskou, 1994; Lagouri et al., 1993). Within this family oregano (*Origanum vulgare* L.) is probably one of most widely used aromatic plant which essential oils are particularly rich in mono- and sesquiterpenes (De Falco et al., 2013).

The genus *Origanum* includes around 38 species, most of which are indigenous to the Mediterranean, Euro-Siberian and Irano-Siberian regions (Sahin et al., 2004). *Origanum vulgare* L. is one of the most widely among all the species within the genus which distributed all over the Europe, West and Central Asia up to Taiwan (Sahin et al., 2004; Radušiene et al, 2008). It is one of the most important culinary herbs in the world. Leaves and flowers of oregano are traditionally used to cure cough and sore throats and for relieve of gastrointestinal disorders. *Origanum vulgare* is a source of essential oils and phenolic metabolites (Radušiene et al, 2008).

The main bioactive components of oregano are phenolic components (Segeit-Kujawa et al., 1990; Kulevanova et al., 2001; Leung & Foster, 2003; Radušienė et al., 2008) and essential oil (Leung & Foster, 2003). *Origanum vulgare* contains 0.1-1.0% of essential oil composed of thymol, carvacrol, β-bisabolene, caryophyllene, *p*-cimene, borneol, linalool, linalyl acetate, geranyl acetate, α-pinene, β-pinene, α-terpinene, with highly variable relative proportion, depending on source (Leung & Foster, 2003).

Oregano essential oils have been shown to possess antioxidant, antibacterial, antifungal, diaphoretic, carminative, antispasmodic and analgesic activities (De Falco et al., 2013). Thymol and carvacrol, usually the major phenols present in oregano, have strong fungicidal, anthelmintic, irritant, and other properties (Leung & Foster, 2003).

Based on numerous studies it was established that oregano essential oil, rich in thymol and carvacrol, has a significant antioxidant activity in the process of the lard oxidation (Lagouri et al., 1993; Tsimidou & Boskou,1994). Yanishlieva and Marinova (1995) examined the antioxidant activity of hexane extracts from oregano grown in Bulgaria, and the mechanism of action of pure thymol and carvacrol (Yanishlieva et al.,1999), while Kulišić and coworker presented different methods for antioxidant activity of oregano essential oil testing (Kulišić et al., 2004).

The aim of the present study was to examine the antioxidant properties of oregano essential oil, originated from Serbia, by using two different methods, namely, DPPH test, and TBA-MDA assay.

Material and methods

Plant material

The commercial sample of aerial parts of *Origanum vulgare* L. (*Origani herba*) was purchased from the local health food store in Leskovac, Serbia. According to the declaration, the material used for investigations originates from Serbia (packed by: MALINA-IMPEX d.o.o. Popučke b.b., Valjevo)

Chemicals and reagent

Ethanol, 96% (Centrochem, Zemun, Serbia), 1,1-diphenyl-2-picrylhydrazyl (DPPH radical), butylated hydroxy toluene (BHT), thiobarbituric acid (TBA), 2,2'-azobis (2-methylpropionamidine) dihydro-chloride (AAPH) (Sigma Chemical Company, St. Louis, USA), trichloroacetic acid (TCA) (J.T. Baker,

VA Deventer, Netherlands). Phospholipids (Phospholipon® 90; PL90) were a gift from Phospholipid GmbH (Cologne, Germany). According to the declaration PL90 mixture consisting of phosphatidylcholine 94.6%, lyso-phosphatidyl-choline 1.3%; fatty acid: palmitic acid 12 ± 2%, stearic acid 3 ± 1%, oleic acid 10 ± 3%, linoleic acid 66 ± 5%, linoleic acid 5% ± 2; peroxide number 1.4.

Isolation of essential oil

Essential oil from aerial parts of *O. vulgare* was isolated by classic Clevenger-type hydrodistillation according to Ph. Jug. V (2000). Plant material (50 g) was immersed in 500 mL of water in round bottom flask, and the oil was isolated using a Clevenger-type apparatus for 180 min. The resulting essential oil was dried over anhydrous sodium sulfate, filtered and stored at +4 °C in a well-filled, airtight container, protected from light, until the analysis.

GC-MS and GC-FID analysis

GC-MS analysis of the oregano essential oil was performed on Agilent Technologies 7890B gas chromatograph, equipped with weakly polar, silica capillary column, HP-5MS (5% diphenyl- and 95% dimethyl-polysiloxane, 30 m x 0.25 mm, 0.25 μm film thickness; Agilent Technologies, USA) and coupled with inert, selective 5977A mass detector of the same company. One μl of the sample dissolved in diethyl ether in the concentration of 1000 ppm was injected in 20:1 split mode. Helium was used as the carrier gas, at a constant flow rate of 1 mL/min. The oven temperature was programmed from 50 °C for 2.25 minutes and then increased to 290 °C at the rate of 4 °C/min. Temperatures of the MSD transfer line, ion source and quadruple mass analyzer were set at 300 °C, 230 °C and 150 °C, respectively. The ionization voltage was 70 eV and mass range m/z 35-650.

GC-FID analysis was carried out under identical experimental conditions as GC-MS. The temperature of the flame-ionization detector (FID) was set at 300°C. Data processing was performed using MSD ChemStation, MassHunter Qualitative Analysis and AMDIS 32 softwares (Agilent Technologies, USA). Retention indices of the components from the analyzed samples were experimentally determined using a homologous series of n-alkanes from C8-C20 as standards. Compounds identification was based on the comparison of their retention indices (RI^{exp} – **Tab. 1**) with those available in literature (Adams, 2007) (RI^{lit} – **Tab. 1**), as well as their mass spectra with those from Willey, NIST and RTLPEST libraries. The percentage composition of particular components in the essential oil was determined on the basis of automatically integrated peak areas of the GC-FID signal.

Antioxidant activity

DPPH assay

Antioxidant activity of oregano essential oil was determined by DPPH test (Aquino et al., 2002; Choi et al., 2002; Sanchez-Moreno, 2002). The essential oil was dissolved in ethanol (96%) and a series of different concentration solutions were prepared (0.098 to 12.5 mg/mL). The ethanol solution of DPPH radical (1 mL, 3×10^{-4} mol/L) was added to 2.5 mL of each essential oil solutions. Absorbance of one sample was immediately measured at 517 nm, while the other samples were incubated at room temperature in the dark, for 20, 30, 45 and 60 minutes, and the absorbance was also measured at 517 nm (A_S). The absorbance at 517 nm was measured for pure ethanol solution of DPPH radical prepared as described above – 1 mL of the DPPH radical (3×10^{-4} mol/L) diluted with 2.5 mL of ethanol, A_C), as well as for the essential oil before treatment with DPPH radical (2.5 mL of essential oil diluted with 1 mL of ethanol, A_B). Free radical scavenging capacity was calculated by the eq. 1 (Stanojević et al., 2015):

$$DPPH\ rsc\ (\%) = 100 - \left[\left(A_S - A_B \right) \cdot \frac{100}{A_C} \right] \quad (1)$$

rsc- radicals scavenging capacity

EC_{50} value was defined as essential oil concentration needed for the neutralization of 50% of the initial DPPH radical concentration. This value was determined by interpolation from the linear regression analysis in the concentration range between 0.098 and 0.391 mg/mL of essential oil added to the reaction mixture. BHT was used as the reference compound.

TBA-MDA assay

Thiobarbituric acid – malondialdehyde (TBA-MDA) test is one of the most commonly used tests for lipid peroxidation process monitoring *in vitro*. It's based on heating of sample with TBA in acidic environment, *i.e.* on TBA and MDA reaction. MDA is end-product of lipid peroxidation formed during hydroperoxide degradation which build pink chromogen ($[TBA]_2$-malondialdehyde adduct) with absorption maximum at 532 nm. Colored complex is formed by condensation of 2 moles of TBA and 1 mole of MDA only from the fatty acid chains that contain at least three double bonds (Halliwell & Chirico, 1993; Laguerre et al., 2007). The antioxidant activity of oregano essential oil was determined by the method developed for caroteinoid,

flavonoids, extracts and some potential antioxidants (Cvetkovic & Markovic, 2008; Cvetkovic et al., 2011; Zvezdanović et al., 2014; Stanojević et al., 2015a), with some modifications. "Sample" contains 0.3 cm^3 of PL90 methanolic solution ($1 \cdot 10^{-2}$ mol/L), and essential oil ethanolic solution (0.042-5.375 mg/mL) in 2:1 (v/v) ratio. Lipid peroxidation was initiated by addition of 0.2 cm^3 of aqueous solution of thermal azo-initiator AAPH ($2.2 \cdot 10^{-2}$ mol/L) during 3 h at a temperature of 40 °C, protected from light. After incubation, 1 mL of aqueous solution of TCA (5.5%), 0.5 mL of methanolic solution of BHT ($1 \cdot 10^{-3}$ mol/L) and 0.5 mL of TBA ($4.2 \cdot 10^{-2}$ mol/L in $5 \cdot 10^{-2}$ mol/L NaOH) were added to the reaction mixture. The mixture was incubated for 10 min at 65 °C and then centrifuged for 5 min at 13800 rpm. The increase in absorbance of the supernatant at 532 nm represents an absorption maximum of generated TBA-MDA complex.

The absorbance of PL90 solution, where lipid peroxidation is initiated with AAPH, treated with TBA (”control“), as well as of PL90 solution, without lipid peroxidation initiation, but also treated with TBA (”blank“). Lipid peroxidation inhibition was calculated by the eq. 2 (Stanojević et al., 2015a):

$$LPI\,(\%) = \frac{(A_C - A_S)}{(A_C - A_B)} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

LPI- Lipid peroxidation inhibition

where A_C – represents the absorbance of ”control“; A_S – absorbance of ”sample“; A_B – absorbance of ”blank“. BHT (0.0125-0.05 mg/mL) was used as the reference compound.

All experiments were carried out in three replications. Data were expressed as mean ± standard deviation. The obtained data were analyzed by Microsoft Excel 2007 and Origin 7 trial.

Results and discussion

Hydrodistillation kinetics and oregano essential oil composition

Figure 1 shows the influence of hydrodistillation time on the essential oil yield. Maximal essential oil yield of 4.1 mL/100g of plant material was achieved after 180 minutes. The hydrodistillation curve shows that there are two different periods of hydrodistillation (**Fig. 1**). The essential oil was evaporated out from the surface from the cells of plant material in the first period (the fast oil hydrodistillation). In the second, slow oil hydrodistillation period, a slow molecular diffusion of the essential oil from internal part from cells of plants material occurred.

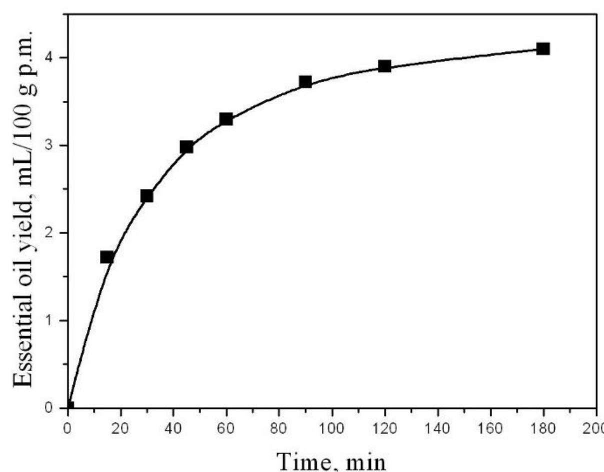

Fig. 1. Hydrodistillation kinetics of oregano essential oil

In the **Tab. 1** and on **Fig. 2** the results of GC–MS analysis of oregano essential oil are presented. Seven components were identified in essential oil: α-thujene, myrcene, α-terpinene, *o*-cymene, γ-terpinene, thymol and carvacrol. The major components were thymol (45%) and carvacrol (37.4%).

The content of thymol and carvacrol in the oregano essential oil from various regions was different: 35% and 32% of thymol and carvacrol, respectively, from Dalmacia (Kulišić et al., 2004), 0.8 and 0.6% respectively, from Turkey (Sahin et al., 2004), 1.1 and 14.3% respectively, from Southern Italy (De Falco et al., 2013), 37.1 and 9.6 respectively, from Iran (Vazirian et al., 2015), 31.8 and 0.2%, respectively from Portugal (Galego et al, 2008). Other investigators reported a lower content of carvacrol in oregano essential oil (less than 35%) compared to the oregano from Serbia.

These changes of thymol and carvacrol content in the oregano essential oil, and changes in the compositions of the oil might arise from several environmental reasons (climatic, seasonal, geographical), used drying method of plant material and genetic differences of the plant material (Faber et al., 1997; Callan et al., 2007; Ghassemi-Golezani et al., 2008; Figiel et al, 2010; Stanojević et al., 2011; De Falco et al, 2013).

Significantly higher content of this two bioactive components with an array of different biological activities is especially important (Ultee et al., 2002; Nostro et al., 2007; Wang et al., 2009; Mathela et al., 2010; Mehdi et al., 2011). The major components are believed to be mainly responsible for the biological activity of this Bakkali, 2008). Based on the results obtained in

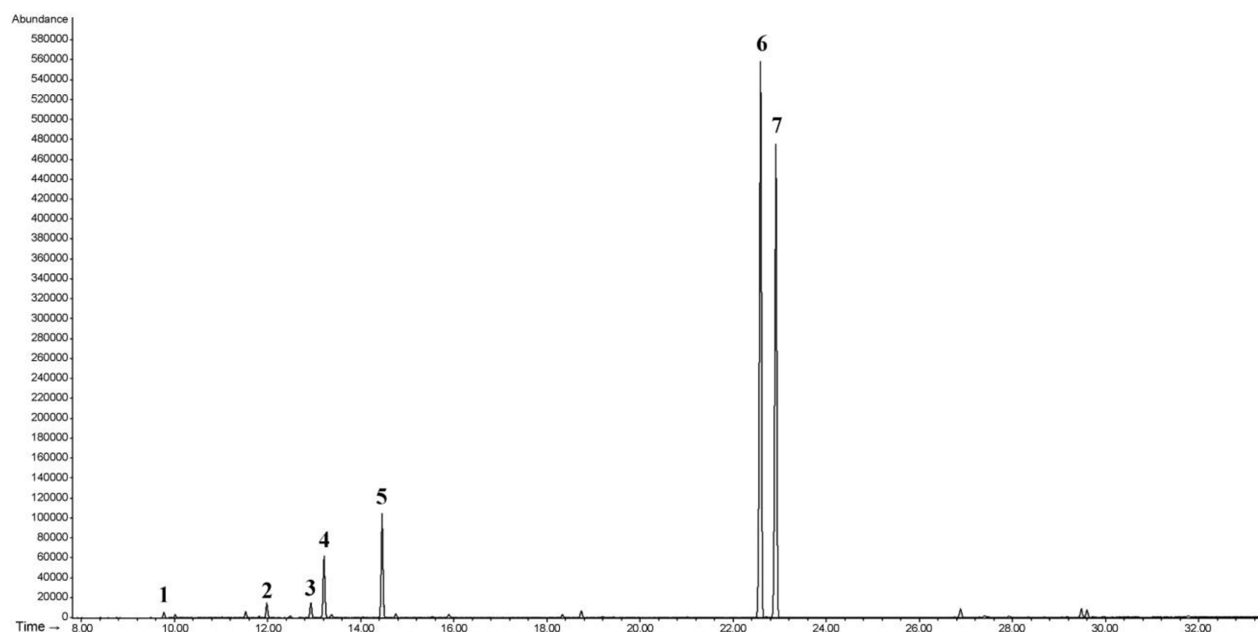

Fig. 2. GC-FID chromatogram of oregano essential oil

Table 1. Chemical composition of *Origanum vulgare* essential oil

No.	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of identification	Content, %
		Monoterpene hydrocarbons (14.4%)				
1	9.78	α-Thujene	925	924	RI, MS	0.4
2	11.99	Myrcene	987	988	RI, MS	1.0
3	12.93	α-Terpinene	1014	1014	RI, MS	1.1
4	13.22	*o*-Cymene	1022	1022	RI, MS	4.4
5	14.46	γ-Terpinene	1057	1054	RI, MS	7.5
		Phenols (82.4%)				
6	22.59	Thymol	1292	1289	RI, MS	45.0
7	22.92	Carvacrol	1300	1298	RI, MS	37.4
					Total	**96.8%**

$t_{ret.}$: Retention time; RI$^{lit\ a,b}$-Retention indices from literature (Adams, 2007), respectively; RI^{exp}: Experimentally determined retention indices using a homologous series of *n*-alkanes (C8-C20) on the HP-5MS column. MS: constituent identified by mass-spectra comparison; RI: constituent identified by retention index matching.

particular essential oil (Bailer et al., 2001; our investigation, the oregano essential oil from Serbia could be a potential natural source of thymol and carvacrol.

Antioxidant activity

DPPH test is most commonly *in vitro* method for antioxidant activities determination of plant extracts and essential oils. It is very suitable and useful for the antioxidant activity determination since it's fast and sufficiently sensitive (Molyneux, 2004). This method is based on hydrogen atoms or electrons exchange between antioxidant molecules and DPPH radicals in the solution (Sanchez-Moreno et al., 2002).

DPPH radical scavenging activity of isolated oregano essential oil was shown on **Fig. 3**, while the EC_{50} values are listed in **Tab. 2**. Degree of DPPH radical neutralization depends on oil concentration as well as the incubation time - it increases with the concentration increase which is the lowest for non incubated samples (**Fig. 3**). The highest antioxidant activity (about 95%) has been measured after 60 minutes of incubation. The EC_{50} (DPPH) value of

synthetic antioxidant BHT was 0.021 mg/mL, indicating better antioxidant activity compared to the tested essential oil. Mentioned antioxidant is the most commonly used synthetic antioxidant, but with harmful effects in human body (I t o et al., 1986). So, presented results suggest that oregano essential oil can be potentially used as a safer alternative to synthetic antioxidants in pharmaceutical and food industries.

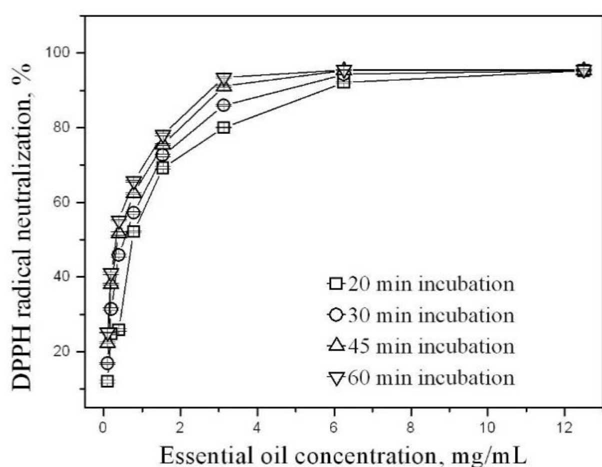

Fig. 3. DPPH antioxidant activity of oregano essential oil

K u l i š i ć et al. (2004) and S a h i n et al. (S a h i n et al., 2004) have come to similar results in their investigations. In earlier reports, thymol and carvacrol, in particular, were found to be main antioxidant components of essential oil from different *Origanum* species (B a r r a t a et al., 1998; R u b e r t o et al., 2002; P u e r t e s - M e j i a et al., 2002). Because the main components of the oregano essential oil in our investigations are phenolics thymol and carvacrol, they are probably mostly responsible for the high degree of DPPH radical neutralization (Y a n i s h l i e v a & M a r i n o v a, 1995; K u l i š i ć et al., 2004; S a h i n et al., 2004).

The TBA method is sensitive and achieves reproducible results. This method is preferable in order to obtain useful data in an environment similar to the real-life situation (K u l i š i ć et al., 2004). **Fig. 4** shows degree of lipid peroxidation inhibition by investigated oregano essential oil, while **Tab. 2** shows the EC_{50} value (TBA-MDA) of the oil.

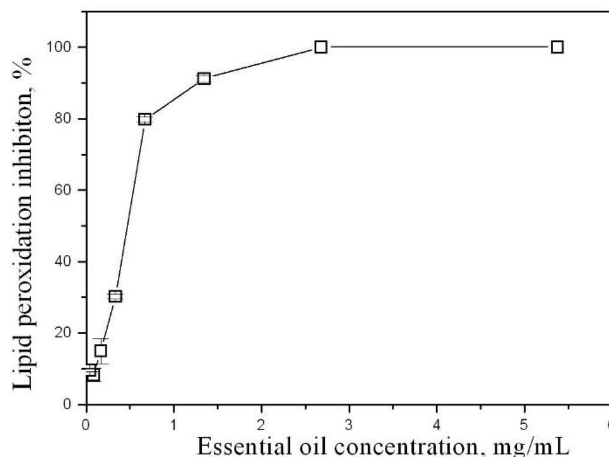

Fig. 4. Inhibition of lipid peroxidation by oregano essential oil (TBA-MDA test)

Based on the presented results it can be concluded that isolated essential oil from oregano shows good concentration dependent antioxidant activity. The highest degree of lipid peroxidation inhibition was achieved by essential oil in concentration of 3.0 mg/mL. The EC_{50} (TBA-MDA) value of synthetic antioxidant BHT was 0.0185 mg/mL.

K u l i š i ć et al. (2004) reported antioxidant activity of oregano essential oil from Dalmacia and its fractions, measured by TBA method. In addition, C a p e c k a et al. (2005) found that *O. vulgare* showed the strongest inhibition of linolenic acid peroxidation.

Our results of antioxidant activity are in accordance with S a h i n et al. (2004) who reported that essential oil of *Origanum vulgare* ssp. *vulgare* possesses antioxidant compounds, and therefore can be used as a natural preservative in food and/or pharmaceutical industry.

Presented results confirm that oregano essential oil possesses remarkable antioxidant activities as assessed by two different methods. This biological effect is probably due to the presence of thymol and carvacrol, but possible synergistic effect

Table 2. EC_{50} values of oregano essential oil

EC_{50} (DPPH), mg/mL			
20 min	30 min	45 min	60 min
0.761 ± 0.003	0.590 ± 0.005	0.360 ± 0.004	0.326 ± 0.002
EC_{50} (TBA-MDA), mg/mL			
0.455 ± 0.004			

among other compounds in the oil can be also suggested.

Conclusion

The presented results indicate that the oregano essential oil could be used as potential source of natural antioxidants for the food, pharmaceutical and chemical industry. Therefore, oregano essential oil represents the alternative to synthetic additives that exhibit toxic and carcinogenic effects. So, it is interesting to investigate its application as natural antioxidant additive in some final food and pharmaceutical products, for preservation and/or extension the shelf-life of raw and processed foods as well as pharmaceuticals. In addition, the results of antioxidant activity in the present study suggested that use of oregano is not just reasonable but it should be even favored in the traditional Serbian cuisine.

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References

Aquino, R., Morelli, S., Tomaino, A., Pellegrino, M., Saija, A., Grumetto, L., Puglia, C., Ventura D., Bonina, F., Grumetto, L. 2002**:** Antioxidant and photoprotective activity of a crude extract of *Culcitium reflexum* H.B.K. Leaves and their major flavonoids. *Journal of Ethnopharmacology*, 79: 183–191.

Bailer, J., Aichinger, T., Hackl, G., Hueber, K., Dachler, M. 2001: Essential oil content and composition in commercially available dill cultivars in comparison to caraway. *Industrial Crops and Products*,14: 229–239.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. 2008: Biological effects of essential oils – a review. *Food and Chemical Toxicology*, 46: 446–475.

Barrata, M.D.S., Dorman, H.J.D., Deans,S.G., Figueiredo, A.C., Barroso, J.G., Ruberto,G. 1998: Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. *Journal of Essential Oil Research*, 10: 618–627.

Bettaieb, I., Bourgou, O., Wannes, W. A., Hamrouni, I., Limam, F. , Marzouk, B. 2010: Essential oils, phenolics, and antioxidant activities of different parts of Cumin (*Cuminum cyminum* L.). *Journal of Agricultural and Food Chemistry*, 58: 10410–10418.

Burt, S. 2004: Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology*, 94: 223–253.

Callan, N.W.,, Johnson, D. L., Westcott, M.P., Welty, L.E. 2007: Herb and oil composition of dill (*Anethum graveolens* L.): Effects of crop maturity and plant density. *Industrial Crops and Products*, 25: 282–287.

Capecka, E., Mareczek, A., Leja, M. 2005: Antioxidant activity of fresh and dry herbs of Lamiaceae species. *Food Chemistry*, 93(2): 223–226.

Choi, W.C., Kim, C.S., Hwang, S.S., Choi, K.B., Ahn, J.H., Lee, Y.M., Park. H.S., Kim, K.S., Lee, Y.M. 2002: Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science,* 163: 1161–1168.

Cvetković, D., Marković, D. 2008: UV-induced changes in anti-oxidant capacities of selected carotenoids toward lecithin in aqueous solution. *Radiation Physics and Chemistry*, 77: 34–41.

Cvetković, D., Marković, D., Cvetković, D., Radovanović, B. 2011: Effects of continuous UV-irradiation on the antioxidant activities of quercetin and rutin in solution in the presence of lecithin as the protective target. *Journal of the Serbian Chemical Society*, 76: 973–985.

De Falco, E., Mancini, E., Roscigno, G., Mignola, E., Taglialatela-Scafati, O., Senatore, F. 2013: Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. *Molecules*, 18: 14948–14960.

Faber, B., Bangert, K., Mosandl, A., 1997: GC-IRMS and enantioselective analysis in biochemical studies in dill (*Anethum graveolens* L.). *Flavour and Fragrance Journal*, 12: 305–314.

Figiel, A., Szumny, A., Gutiérrez-Ortíz, A., Carbonell-Barrachina, A.A. 2010: Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method. *Journal of Food Engineering*, 98: 240–247.

Galego, L., Almeida, V., Gonçalves, V., Costa, M., Monteiro, I., Matos, F., Miguel, G. 2008: Antioxidant activity of the essential oils of *Thymbra capitata*, *Origanum vulgare*, *Thymus mastichina* and *Calamintha baetica*. *Acta Horticulture*, 765:325-34.

Ghassemi-Golezani, K., Andalibi, B., Zehtab-Salmasi, S., Saba, J. 2008: Effects of water stress during vegetative and reproductive stages on seed yield and essential oil content of dill (*Anethum graveolens* L.). *Journal of Food*, *Agriculture and Environment,* 6: 282–284.

Halliwell, B., Chirico, S. 1993: Lipid peroxidation: its mechanism measurement, and significance. *American Journal of Clinical Nutrition*, 57: 715S-725S.

Ito, N., Hirose, M., Fukushima, H., Tsuda, T., Shirai, T., Tatenatsu, M. 1986: Studies on antioxidants: their carcinogenic and modifying effects on chemical carcinogens. *Food and Chemical Toxicology*, 24:1071–1092.

Kulevanova, S., Stefova, M., Stefkov, G., Stafilov. T. 2001: Identification, isolation, and determination of flavones in *Origanum vulgare* from Macedonian flora. *Journal of Liquid Chromatography & Related Technologies*, 24(4): 589–600.

Kulišić, T., Radonić, A., Katalinić, V., Miloš, M. 2004: Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, 85: 633–640.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., Boskou, D. 1993: Composition and antioxidant activity of essential oils from Oregano plants grown wild in Greece. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 197: 20–23.

Laguerre, M., Lecomte, J., Villeneuve, P. 2007: Evaluation of the ability of antioxidants to counteract lipid oxidation: existing methods, new trends and challenges. *Progress in Lipid Research*, 46: 244–282.

Leung, A.Y., Foster, S. 2003: Encyclopedia of common natural ingredients (used in food, drugs, and cosmetics), Second Edition, A John Wiley & Sons, Inc., Hoboken, New Jersey. 398–399.

Maestri, D.M., Nepote, V., Lamarque, A.L., Zygadlo, J.A. 2006. Natural products as antioxidants. In: Imperato F. (ed.), Phytochemistry: advances in research, Research Signopost: 105–135, Kerala, India.

Mathela, C. S., Singh, K. K., Gupta, V. K. 2010: Syntesis and in vitro antibacterijal activity of thymol and carvacrol derivates. *Acta Poloniae Pharmaceutican Drug Research*, 67(4): 375–380.

Mehdi, S.J., Ahmad, A., Irshad, M., Manzoor, N., Rizvi, M.M.A. 2011: Cytotoxic effect of Carvacrol on human cervical cancer cells. *Biology and Medicine*, 3(2): 307–312.

Molyneux, P., 2004: The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2): 211–219.

Nostro, A., Sudano Roccaro,A., Bisignano, G., Marino, A., Cannatelli, M.A., Pizzimenti, F.C., Cioni, P.L., Procopio. F. and Blanco, A.R. 2007: Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *Journal of Medical Microbiology*, 56: 519–523.

Pharmacopoeia Jugoslavica V, 2000: Savremena administracija, Beograd (in Serbian), Vol. 1: 118.

Puertes-Mejia, M., Hillebrand,S., Stashenko,E., Winterhalter, P. 2002: In vitro radical scavenging activity of essential oils of Columbian plants and fractions from oregano (*Origanum vulgare* L.) essential oil. *Flavour and Fragrance Journal*, 17: 380–384.

Radušienė, J., Ivanauskas, L., Janulis, V., Jakštas, V. 2008: Composition and variability of phenolic compounds in *Origanum vulgare* from Lithuania. *Biologia*, 54(1): 45–49.

Ruberto, G., Barrata, M.T., Sari, M., Kaabexhe, M. 2002: Chemical composition and antioxidant activity of essential oils from Algerian *Origanum glandulosum* Desf. *Flavour and Fragrance Journal*, 17: 251–254.

Sahin, F., Gulluce, M., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M., Agar, G., Ozer, H. 2004: Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. *Food Control*, 15: 549–557.

Sanchez-Moreno, C. 2002: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International*, 8(3), 121–137.

Segeit–Kujawa, E., Michalowska, A. 1990: Determination of flavonoids in Hb. Origani. *Herba Polonica*, 36(3): 79–82.

Stanković, M.Z., Stanojević, LJ.P., Tehnologija lekovitog i začinskog bilja, Tehnološki fakultet, Leskovac, 2014. 113 p.

Stanojević, J.S., Stanojević, Lj.P., Cvetković, D.J., Danilović B.R., 2015: Chemical composition, antioxidant and antimicrobial activity of the turmeric essential oil (*Curcuma longa* L.). *Advanced technologies*, 4(2): 19–25.

Stanojević, Lj.P., Stanojević, J.S., Cvetković, D. J., Cakić, M.D., Ilić, D.P. 2015a: Antioksidativna aktivnost etanolnog ekstrakta lista gajene jagode (*Fragariae folium*). *Hemijska Industrija*, 69(5): 567–576.

Stanojević, Lj., Stanković, M., Cakić, M., Nikolić, V., Nikolić, Lj., Ilić, D., Radulović, N. 2011: The effect of hydrodistillation techniques on yield, kinetics, composition and antimicrobial activity of essential oils from flowers of *Lavandula officinalis* L. *Hemijska Industrija*, 65: 455–463.

Tongnuanchan, P., Benjakul, S. 2014: Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science*, 79(7): R1231–R1249.

Tsimidou, M., Boskou, D. 1994: Antioxidant activity of essential oils from the plants of the Lamiaceae family. In: G. Charalambous, Spices, herbs and edible fungi, 273–284, Amsterdam: Elsevier.

Ultee, A., Bennik, M. H. J., Moezelaar, R. 2002: The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, 68(4):1561–1568.

Vazirian, M., Mohammadi, M., Farzaei, M.H., Amin, G., Amanzadeh, Y. 2015: Chemical composition and antioxidant activity of *Origanum vulgare* subsp. *vulgare* essential oil from Iran. *Research Journal of Pharmacognosy*, 2(1): 41–46.

Wang, Q., Gong, J., Huang, X., Yu, H., Xue. F. 2009: In vitro evaluation of the activity of microencapsulated carvacrol against *Escherichia coli* with K88 pil. *Journal of Applied Microbiology* 1, 107(6): 1781–178.

Yanishlieva, N.V., Marinova, E.M., Gordon, M.H., Raneva, V.G. 1999): Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chemistry*, 64: 59–66.

Yanishlieva, N.V., Marinova, E.M. 1995: Antioxidant activity of selected species of the family Lamiaceae grown in Bulgaria. *Die Nahrung*, 39: 458–463.

Zvezdanović, J., Daskalova, L., Yancheva, D., Cvetković, D., Marković, D., Anderluh, M., Šmelcerović, A. 2014: 2-Amino-5-alkylidenethiazol-4-ones as promising lipid peroxidation inhibitors. *Monatshefte Fur Chemie*,145: 945–952.