

Original Article

Received: 18 January 2015

Revised: 19 February 2016

Accepted: 01 Mart 2016

Dill (*Anethum graveolens L.*) seeds essential oil as a potential natural antioxidant and antimicrobial agent

Ljiljana P. Stanojević*, Mihajlo Z. Stanković, Dragan J. Cvetković, Bojana R. Danilović, Jelena S. Stanojević

University of Niš, Faculty of Technology, Bulevar Oslobođenja 124, 16000 Leskovac, Serbia

* E-mail: stanojevic@tf.ni.ac.rs

Abstract:

Stanojević, Lj.P., Stanković, M.Z., Cvetković, D.J., Danilović, B.R., Stanojević, J.S.: Dill (*Anethum graveolens L.*) seeds essential oil as a potential natural antioxidant and antimicrobial agent. *Biologica Nyssana*, 7 (1), September 2016: 31-39.

Synthetic antioxidants and antimicrobial agents can induce many undesired side effects, which attracts interest of food producers and consumers in finding ingredients of natural origin. The antioxidative and antimicrobial activity of essential oil from dill (*Anethum graveolens L.*) seeds was investigated in terms of its possible application as natural antioxidant and antimicrobial agent. DPPH test and FRAP method have been used for the investigation of antioxidative activity of essential oil. Disc-diffusion method has been used for investigation of oil antimicrobial activity on following microorganisms: *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella enteritidis* and *Candida albicans*. Essential oil, in concentration of 29 mg/mL, incubated for 60 minutes has shown the highest degree of DPPH radicals' neutralization (79.62%). FRAP activity of oil was 40.63 $\mu\text{mol Fe}^{2+}/\text{g}$ of essential oil. Essential oil showed the best antimicrobial activity on *Staphylococcus aureus*. Furthermore, there was a significant antimicrobial activity on all investigated microorganisms.

Key words: *Anethum graveolens L.*, dill seeds, essential oil, antioxidant activity, antimicrobial activity

Apstrakt:

Stanojević, Lj.P., Stanković, M.Z., Cvetković, D.J., Danilović, B.R., Stanojević, J.S.: *Etarsko ulje semena mirođije (Anethum graveolens L.) kao potencijalni prirodni antioksidans i antimikrobni agens. Biologica Nyssana*, 7 (1), Septembar 2016: 31-39.

Sintetski antioksidansi i antimikrobni agensi mogu dovesti do brojnih neželjenih efekata, pa je zato sve veće interesovanje proizvođača i potrošača hrane za sastojcima prirodnog porekla. Proučavana je antioksidativna i antimikrobna aktivnost etarskog ulja semena mirođije (*Anethum graveolens L.*) u cilju moguće primene kao prirodnog antioksidansa i antimikrobnog agensa. Antioksidativna aktivnost etarskog ulja je određena primenom DPPH-testa i FRAP metode. Antimikrobna aktivnost ulja je određena disk-difuzionom metodom, na sledeće mikroorganizme: *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella enteritidis* i *Candida albicans*. Najveći stepen neutralisanja DPPH radikala (79,62%) pokazuje ulje inkubirano 60 minuta, u koncentraciji 29 mg/mL. FRAP vrednost etarskog ulja iznosi 40,63 $\mu\text{mol Fe}^{2+}/\text{g}$ etarskog ulja. Ulje pokazuje najbolje antimikrobno dejstvo na *Staphylococcus aureus*. Takođe, postoji značajna antimikrobna aktivnost na sve ispitivane mikroorganizme.

Key words: *Anethum graveolens L.*, seme mirođije, etarsko ulje, antioksidativna aktivnost, antimikrobna aktivnost

Introduction

There are an increasing number of scientific investigations to find natural products that exhibit different biological activities, and antioxidant, antimicrobial and anti-inflammatory activities are the most commonly studied (Tepe et al., 2004; Bakkali et al., 2008; Mišić et al., 2008).

One of the most important trends in food industry is discovery of natural antioxidants from plant material (De laquis, 2002). One of the most efficient ways of lipid peroxidation inhibition is the addition of synthetic antioxidants to the oils and foods, such as ascorbyl palmitate (AP), *tert*-butyl-4-hydroxyanisole (BHA), *tert*-butyl-4-hydroxytoluene (BHT), propyl gallate (PG), butyl gallate (BG), octyl gallate (OG), dodecyl gallate (DG). But, synthetic antioxidants have some undesirable side effects wherefore natural antioxidants are increasingly used (Maestri et al., 2006).

Plants have long been used for various infectious diseases treatment and some of these traditional medicines are still involved in the treatment of various diseases (Mendonça-Filho, 2006). Essential oils and herbal extracts, as sources of natural products, have become interesting in recent decades. They represent an alternative to synthetic antioxidants and antimicrobial agents in food industry (Tepe et al., 2004; Hinneburg et al., 2006) as well as in pharmaceutical industry, alternative medicine and natural therapy (Burt, 2004; Tepe et al., 2004; Mišić et al., 2008).

Anethum graveolens L., commonly known as dill, is an annual and sometimes biennial medicinal plant from the family Apiaceae (Umbelliferae). Dill is one of the most significant spices in food industry (Orhan et al., 2013; Leung & Foster, 2003). Dill is native plant to Mediterranean region, southeastern Europe and central southern Asia (Kaur & Arora, 2010). Dill herb and dill seeds have been used as flavoring agent in food industry for sauces, salads and seafood (Pino et al., 1995; Kaur & Arora, 2010). The food industry often uses essential oil instead of dill leaves and seeds (Pino et al., 1995) due to its characteristic aroma and flavor (Jirovetz et al., 2003). It has been reported that dill has antimicrobial, antihyperlipidemic, diuretic, hypotensive, antispasmodic, antiemetic, laxative effect (Koppula & Choi, 2011; Hosseinnzadeh et al., 2002; Tucakov, 1997) and anticancer activity (Peerakam et al., 2014). Bioactive components of dill are: essential oil, fatty oil, proteins, carbohydrates, fiber, mineral elements (potassium, calcium, magnesium, phosphorous, sodium), vitamin

A and niacin (Kaur & Arora, 2010). Essential oil is present in all parts of plant, but its content is the highest in the seeds (2-5%) (Leung & Foster, 2003). The major component in dill seeds essential oil is carvone (20-60%) (Leung & Foster, 2003; Radulescu et al., 2010, De laquis et al., 2002). Besides carvone, there are also present: limonene, α -phelandrene, α -pinene, α -terpinene, apiole, dill apiole, 1,8-cineole, dihydro carvone and *p*-cymene (Leung & Foster, 2003; Pino et al., 1995). In their study, Stanojević et al. (2015) and coworkers have found a high content of carvone (about 90%) in dill seeds essential oil from the territory of Southeast Serbia.

Since dill seeds are one of the most commonly used spices in Serbian traditional cuisine and food industry, and, at the same time, dill is a plant with many medicinal properties, the aim of this study was to investigate antioxidant activity of essential oil from dill seeds by two antioxidant assay: DPPH and FRAP as well as antimicrobial activity against some intestinal pathogens.

Material and methods

Plant material

The commercial sample of non-disintegrated dill seeds (*Anethi fructus*) was purchased („Planta Mell“, Svrlijig, Southeast Serbia) and used for investigations.

Chemicals and reagents

Ethanol, 96% (Centrochem, Zemun, Serbia), 2,4,6-Tris (2-pyridyl)-1,3,5-triazine (TPTZ reagent), 1,1-diphenyl-2-picrylhydrazyl (DPPH radical), butylated hydroxy toluene (BHT), iron (III) chloride hexahydrate, iron (II) sulfate heptahydrate (Sigma Chemical Company, St. Louis, USA), dimethyl sulfoxide (DMSO; BDH, Milan, Italy). All other chemicals were analytical-grade.

Isolation of essential oil

Essential oil from dill seeds was isolated by classic Clevenger-type hydrodistillation (cohobation) according to Ph. Jug. V (2000). Dill seeds (15 g) were immersed in 300 mL of water in round bottom flask, and the oil was isolated using a Clevenger-type apparatus for 3 h. The obtained essential oil was dried over anhydrous sodium sulfate and used for analysis (Stanojević et al., 2015).

Antioxidant activity

DPPH assay

Antioxidant activity of essential oil was determined by the use DPPH test (Aquinio 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 (0.23 to 29 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_U). 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_K), 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 following equation (Stanojević et al., 2015a):

$$\text{DPPH radicals scavenging capacity (\%)} = 100 - \left[(A_U - A_B) \cdot \frac{100}{A_K} \right] \quad (1)$$

Essential oil concentration needed for the neutralization of 50% of the initial DPPH radical concentration is called EC_{50} value. This value was determined by interpolation from the linear regression analysis in the concentration range between 0.23 and 29 mg/mL of essential oil added to the reaction mixture. BHT was used as the reference compound ($EC_{50} = 0.021$ mg/mL).

FRAP assay

The antioxidant activity of essential oil by FRAP assay is determined using Benzie and Strain method with some modifications (Benzie & Strain, 1996). FRAP reagent was prepared from acetate buffer (300 mmol/L, pH = 3.6), TPTZ reagent (10 mmol/L in 40 mmol/L HCl) and $FeCl_3 \cdot 6 H_2O$ (20 mmol/L) in 10:1:1 ratio.

Ethanol solution of essential oil (0.1 mL, concentration 9.25 mg/mL) and 3 mL of FRAP reagent were added in a test tube. Absorbance was measured at 593 nm after 30 minutes of incubation at 37 °C against blank control. The calibration curve for FRAP values determination was obtained by measuring the absorbance of Fe^{2+} (0.2 to 1 mmol/L $Fe_2SO_4 \cdot 7H_2O$) standard solution, which was treated in the same way as the essential oil samples. FRAP value was expressed as $\mu\text{mol } Fe^{2+}/\text{g}$ of the essential oil.

Antimicrobial activity

Microorganisms and substrates. Six microorganisms were selected to determine the antimicrobial activity: *Staphylococcus aureus* (ATCC 25923), *Bacillus*

subtilis (ATCC 6633), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 19166), *Salmonella enteritidis* (ATCC 13076) and *Candida albicans* (ATCC 10259). Media used for the growth of the microorganisms: Antibiotic agar no. 1 for microbiology (Merck, Darmstadt, Germany) for bacteria and Sabouraud dextrose agar (Torlak, Belgrade) for fungi. Microorganisms are from the collection of the Microbiological laboratory of the Faculty of Technology, Leskovac.

Disc-diffusion method. The agar disc-diffusion method was used for testing antimicrobial activity of dill seeds essential oil (Kiehlbauch et al., 2000). The mediums were sterilized for 15 minutes in an autoclave at 121°C. The suspension was prepared with overnight culture and adjusted to 0.5 McFarland standard. The inoculum of 0.1 ml of suspension was added to 10 mL of medium and poured into the Petri dishes.

For screening, sterilized filter paper disks (12.7 mm dia., Schleicher & Schuell) were placed on the surface of inoculated mediums and impregnated with 60 μl of essential oil. Plates were incubated for 24 hours at 37 °C for bacteria, and 48 hours at 25 °C for yeast. Antimicrobial activity was expressed as the diameter of inhibition zones (mm) obtained by investigated sample.

Standardized discs of Amoxicillin (30 $\mu\text{g}/\text{disc}$, Hemofarm, A.D. Vršac), Cephalexin (30 $\mu\text{g}/\text{disc}$, Panfarma, Beograd), Amracin (30 $\mu\text{g}/\text{disc}$, Galenika, A.D. Zemun) and Nystatin (100 U/disc, Bioanalyse) served as positive controls.

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

Results and discussion

Essential oil composition

The moisture content and initial oil content in dill seeds were 7.33% and 4.0 mL/100 g of dry plant material, respectively. The yield of essential oil was 2.80 mL/100 g dry plant material (Stanojević et al., 2015).

In the essential oil twenty nine components have been identified (99.9% of all components). These results are presented in our previous studies where the influence of the technique on the yield, composition and kinetics of essential oil hydrodistillation from dill seeds have been investigated (Stanojević et al., 2015).

It has been found by GC-MS analysis of essential oil that carvone has the highest content (85.9%). Carvone content in dill essential oil is usually 20 to 60% (Leung & Foster, 2003; de

Carvalho et al., 2006). Besides carvone, limonene (5.1%), *cis*-dihydrocarvone (3.0%), *trans*-dihydrocarvone (2.7%), *cis*-carveol (1.8%) and *trans*-carveol (1.4%), all other components of oil were identified in much lower concentrations (Stanojević et al., 2015).

The higher content of carvone in oil from Serbia compared to its content in oils from other areas (Bulgaria, Canada, India and Romania) (De laquis et al., 2002; Jirovetz et al., 2003; Singh et al., 2005) is probably due to different climatic conditions, as well as genetic characteristics of seeds (Stanojević et al., 2015).

Antioxidant activity

Antioxidant activity of essential oil investigated by DPPH test (ability of oil in different concentrations to scavenge free DPPH radical) is shown on Fig. 1 (results for not-incubated and 20, 30, 45 and 60 min incubated samples are represented).

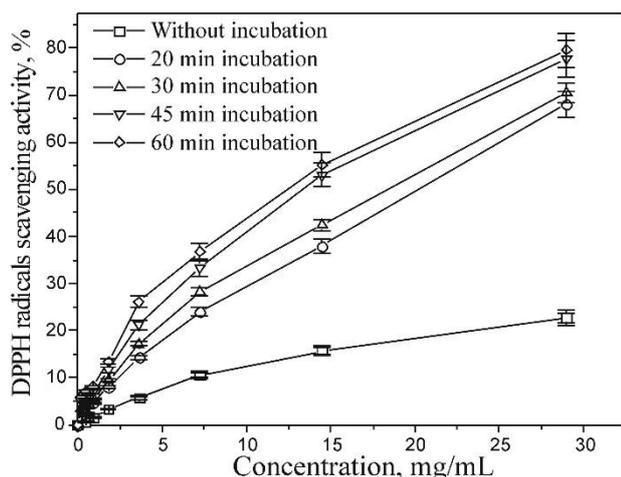


Fig. 1. DPPH radicals scavenging activity by dill (*Anethum graveolens* L.) seeds essential oil

It can be noticed that the degree of DPPH neutralization depends on incubation time, for all investigated concentrations of oil. The highest degree of DPPH radicals' neutralization is for 60 minutes incubation, in concentration of 29 mg/mL (79.62%).

EC₅₀ values of essential oil are shown in Tab. 1. Non-incubated samples of essential oil have not achieved EC₅₀ value in the investigated range of concentrations. The tested synthetic antioxidant has showed better antioxidant activity compared to the essential oil. EC₅₀ values of essential oils are lower than the EC₅₀ values of synthetic antioxidant, BHT. These synthetic antioxidants are used in food industry despite their documented undesirable side effects (Tepe et al., 2005). Based on these results (Fig. 1 and Tab. 2) it can be concluded that the incubation time has effect on DPPH radicals'

neutralization. Dill seeds essential oil from Thailand has shown lower extent of DPPH neutralization (EC₅₀ = 128.49 mg/mL) than oil obtained in our investigation (Nanasombat & Wittigsoil, 2011).

Table 1. EC₅₀ values of dill (*Anethum graveolens* L.) seeds essential oil

Incubation time, min	EC ₅₀ , mg/mL*
20	20.27 ± 0.811
30	18.20 ± 1.019
45	13.45 ± 0.807
60	12.20 ± 0.464

*All data represent the mean of tree replications ± standard deviation (Mean ± SD).

Removal of free radicals is very important in food and food products preservation (Hinneburg et al., 2006; Tepe et al., 2004). Essential oil of dill seeds can be an alternative to dill as spice in the form of powdered plant (whole or some parts). Oil is also a potential source of natural antioxidants, as a possible alternative to synthetic antioxidants. But, for this potential application of dill oil it is necessary to perform *in vivo* tests, which will be the aim of our further investigation.

Essential oil of dill seeds has lower DPPH neutralization activity compared to acetone extracts of dill seeds (Singh et al., 2005). Better activity of extract compared to unstable oil is probably due to presence of nonvolatile phenol compounds. In addition, some of the compounds with a different polarity, which are present in very small amounts in the extract, are also able to contribute to better antioxidative activity of extract. Some compounds can originate in extract during hydrolysis or other processes of decomposition. Some chemical reactions initiated by heating can also drive up to activities changes of complex extract, composed of a number of compounds with different chemical and physical properties (Singh et al., 2005).

Sintim et al. (2015) reported on the significant effect of the antioxidant capacity of the dill seed essential oil using the oxygen radical absorbance capacity (ORAC) method.

In addition, it was found that dill essential oil from the aerial parts of the plant had antioxidant activity. Kazemi reported that dill oil exhibited a high activity in each antioxidant system with a special attention for β-carotene bleaching test and reducing power (Kazemi, 2015).

FRAP value, as a measure of essential oil antioxidant activity, was 40.63 μmol Fe²⁺/g of essential oil. Lado et al. (2004) determined the antioxidant properties of commercially purchased essential oils using the FRAP assay (cumin,

coriander, dill, chamomile, hyssop, lavender, parsley, rosemary, sage and yarrow). FRAP value of the investigated dill essential oil was 42.64 $\mu\text{mol/g}$. The essential oil, investigated in our work, was incubated for 30 min at 37 °C with FRAP reagent, while Lado et al. (2004) have been incubated the oil only for 5 minutes. Due to the long incubation time our oil should express greater FRAP value. However, lower FRAP value of our essential oil is probably the result of different chemical composition compared to the oil used by Lado and coworkers. Different origin of plant material can be also the reason of such differences in obtained results. **Tab. 2** shows the FRAP values of dill essential oil as well as literature FRAP values of some volatile components of oil (carvone, limonene and linalool).

Table 2. FRAP value of dill (*Anethum graveolens* L.) seeds essential oil as well as literature FRAP values of some volatile oil components

Essential oil or volatile component	FRAP value, $\mu\text{mol Fe}^{2+}/\text{g}$ essential oil
Dill seeds essential oil	40.63 \pm 3.23*
Dill seeds essential oil ^a	42.64 ^a
Carvone ^a	15.77 ^a
Linalool ^a	25.73 ^a
Limonene ^a	37.44 ^a

^aLado et al. (2004)

*All data represent the mean of three replications \pm standard deviation (Mean \pm SD).

The dill essential oil has the highest FRAP value (**Tab. 2**), but the individual components identified in the essential oil also have the reductive capacities (Lado et al., 2004). FRAP value obtained in our study is most likely the result of reducing ability of components present in the oil, especially carvone and limonene, which are present in the highest content. Such activity of oil is due to the presence of minor component too, such as dihydrocarvone, *cis*- and *trans*-carveol and linalool. So, FRAP value of oil is a result of synergistic effect of all present components since reducing ability of oil is higher than reducing ability of individual components (oil shows the highest FRAP activity). A significant number of natural products investigations suggest that essential oils have antioxidant activity. It is believed that antioxidants are directly responsible for antimutagenic and anticarcinogenic activity due to their radical scavenging properties. Essential oil with antioxidant activity could be beneficial for human health (Bakkali et al., 2008).

Antimicrobial activity

Antimicrobial activity of dill essential oil as well as activity of reference antibiotics are shown in **Tab. 3**.

Essential oil of dill has effect on all tested microorganisms. The highest effect was observed on *S. aureus*. Tested antibiotics showed much less activity on this bacterium. It has also a significant effect on *B. subtilis* and *E. coli*.

Staphylococcus aureus is a gram-positive bacterium that is, among many other harmful effects to humans, one of the most frequent mastitis agents in herds of dairy cows (Sing & Prakash, 2008; Milanov et al., 2010). Milk contaminated with *S. aureus*, as well as products from such milk can cause a variety of infections, by bacteria itself and by their enterotoxins (Samaržija et al., 2007). The relatively high effect of dill essential oil upon these bacteria suggests the potential use of oil as a natural antimicrobial agent in milk products. However, in order to use the obtained oil in such way, detailed studies are necessary to establish its minimum inhibitory concentration as well as its acute toxicity in particular concentration which would be the goals of our further studies.

It is significant that investigated essential oil has a higher effect on *B. subtilis* compared to widely used Cephalixin and Amoxicillin antibiotics. *Bacillus* species most likely cause alimentary toxic infections in humans. Toxic infections are consequence of various food products consumption, in which starch and proteins are dominating, such as rice, meat and meat products, desserts, and other canned food. They are very often present as contaminants in food of animal and vegetable origin since they can survive various physical and chemical conditions because of resistant spores. In addition to alimentary infection, *Bacillus* causes a number of other diseases: septic meningitis, cellulitis, gangrene, and many eye infections (Kotironta et al., 2000). Based on this results it can be concluded that dill seeds essential oil can be used for natural antimicrobial formulation production.

Essential oil from dill seeds shows higher antimicrobial activity on *E. coli* (zone diameter 38 mm) compared to Cephalixin and Amoxicillin antibiotics. *E. coli* is considered as a dominant bacterium species in the digestive tract. The presence of this bacterium in water and food is a reliable indicator of fecal contamination. This bacterium commonly contaminates meat and dairy products, as well as fruit and vegetables (Markov et al., 2009). Presence of enteropathogenic *E. coli* in the food products can cause vomiting and diarrhea in infants and young children (Singh & Prakash, 2008).

Table 3. Antimicrobial activity of dill (*Anethum graveolens* L.) seeds essential oil

Microorganism	Essential oil	Antibiotic			
		Cephalexin	Amracin	Amoxicillin	Nystatin
Inhibition zone diameter, mm					
<i>Escherichia coli</i>	38.0 ± 1.52	26.0 ± 1.04	n.t.	28.0 ± 0.84	n.t.
<i>Staphylococcus aureus</i>	80.0 ± 2.16	26.0 ± 0.78	n.t.	27.0 ± 0.97	n.t.
<i>Salmonella enteritidis</i>	28.0 ± 0.97	30.0 ± 1.08	25.0	31.0 ± 0.90	n.t.
<i>Listeria monocytogenes</i>	20.0 ± 0.83	34.0 ± 0.88	n.t.	36.0 ± 0.72	n.t.
<i>Bacillus subtilis</i>	52.0 ± 1.38	48.0 ± 0.96	52.0 ± 0.99	37.0 ± 0.59	n.t.
<i>Candida albicans</i>	18.0 ± 0.57	n.t.	n.t.	n.t.	17.0 ± 0.32

n.t.-not treated; All data represent the mean of tree replications ± standard deviation (Mean ± SD).

The good antimicrobial activity of essential oil against *E. coli* probably mainly originates from carvone, which is present in the amount of 86% in the oil. This result is in accordance with studies of Naigre and coworkers who determined the effect of carvone on *Enterococcus faecium*, *Escherichia coli* and *Aspergillus niger* (Naigre et al., 1996).

Salmonella are gram-negative bacteria from *Enterobacteriaceae* family and they are the most common causes of food poisoning. *Salmonella* are natural inhabitants of animals' gastrointestinal tract; they are widespread in soil, water and plants. All representatives of *Salmonella* genus are potential human pathogens. They cause three types of disease in humans - enteric fever, sepsis and gastroenteritis. About 95% of *Salmonella* are ingested through food and the most common sources of infection are milk and dairy products, eggs, meat and meat products (Cox, 2000; Markov et al., 2009). There are scientific papers on antimicrobial activity of commercial dill seeds essential oil on *Salmonella typhimurium* (De laquis et al., 2002). There is no data about the effect of dill seeds essential oil from the Southeast Serbia region on *Salmonella* species. The investigated dill seeds oil had a significant effect against *S. enteritidis* which is in the range with the effect of the commercial antibiotics.

L. monocytogenes is a pathogenic bacterium that leads to listeriosis disease after consumption of food. This is a particularly dangerous pathogen since it can survive at low temperatures (foods that are kept in the fridge). Listeriosis is one of the most common diseases with a fatal outcome (30%) (Markov et al., 2009). There are studies of essential oils effects on *L. monocytogenes* (De laquis et al., 2002). Dill seeds essential oil has less effect on bacteria compared to fractions of oils that are rich in carvone and limonene (De laquis et al., 2002). Carvone, as

a major component of the oil isolated in our study exhibited antimicrobial activity against *L. monocytogenes* (de Carvalho et al., 2006), so it is probably the most responsible for the effect of oil on these bacteria.

Isolated essential oil showed antifungal activity against *C. albicans*, which is, again, most probably due to the high content of carvone in the oil. Carvone and limonene are the main components of caraway essential oil which showed strong antifungal activity against *C. albicans*. Limonene is a carvone precursor and it is mainly present in *Mentha* species being toxic to most microorganisms. Carvone is widely used in the manufacture of aromas and fragrances and it has antifungal activity (Pina et al., 2012).

Commercial dill seeds essential oil, with content of limonene and carvone of 46.3% and 49.5% respectively, shows antimicrobial activity against *Pseudomonas fragi*, *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*. The fractions of essential oils with high content of limonene (more than 90%), and fractions with high content of carvone (67-99%) have shown better activity against gram-positive and gram-negative bacteria. Thereby, the fractions rich in carvone have showed weaker activity in some extents compared to fractions rich in limonene (De laquis et al., 2002).

Essential oils are complex mixtures and their biological properties are result of synergistic effects of all components or major compounds. In most cases, biological activity of main components only, like thymol, carvacrol, linalool, terpineol, eugenol, carvone, geraniol, citronellol, nerol, safrole, eucalyptol, limonene, cinnamaldehyde, were analyzed. Generally, the main components of

essential oils are the ones on which biophysical and biological properties of oils depend (Bakkali et al., 2008).

Antimicrobial activity of dill seeds essential oil in our study probably originates from carvone, having in mind literature data about antimicrobial effect of carvone on a large number of bacteria and fungi (Singh et al., 2005). There are also data of limonene antimicrobial activity, a component that is represented with about 5% in isolated oil in this study (Deliquis et al., 2002; Singh et al., 2005). Other components of the oil probably also contribute to this activity. The high content of carvone, monoterpenic ketone, with numerous biological properties, indicates the possible application of dill seeds essential oil for medical purposes beside food industry, as a bioactive product of natural origin. Antimicrobial activity of dill seeds essential oil is significant for human and animal pathogens as well as for food protection (Bakkali et al., 2008).

Conclusion

Essential oils are the source of natural products with different pharmacological activities. These oils represent the complex mixture of number components what is the reason for difficult explanation of their pharmacological activities. The presented data on antimicrobial and antioxidant activities of dill seeds essential oil showed that the isolated oil (Southeast Serbia, Svrlijig), with a high content of carvone is a potential source of natural antioxidants and antimicrobial agents. The results indicate possible application of essential oils in food and pharmaceutical industry as a safer alternative to synthetic antioxidants and antimicrobial agents. Bearing in mind the results obtained in this work, dill seeds essential oil should be considered for further investigation with practical applications in different food and pharmaceutical systems.

Acknowledgements. This research is a part of Project TR-34012 which is supported by Ministry of Education, Science and Technological Development of the Republic of Serbia.

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.
- Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. 2008: Biological effects of essential oils - a review. *Food and Chemical Toxicology*, 46 (2): 446–475.
- Benzie, I.F.F., Strain, J.J. 1996: The ferric reducing ability of plasma (Frap) as a measure of "Antioxidant Power": The Frap Assay. *Analytical Biochemistry*, 239 (1): 70–76.
- Burt, S. 2004: Essential oils: their antibacterial properties and potential application in foods – a review. *International Journal of Food Microbiology*, 94 (3): 223–253.
- 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.
- Cox, J. 2000. Salmonella. In: Robinson, R.K., Batt, C.A., Patel, P.D. (eds.), *Encyclopedia of food microbiology: 1928–1937*. Academic Press, London.
- de Carvalho, C.C.C.R., da Fonseca, M.M.R. 2006: Carvone: Why and how should one bother to produce this terpene. *Food Chemistry*, 95 (3): 413–422.
- Deliquis, P.J., Stanich, K., Girard, B., Mazza, G. 2002: Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology*, 74 (1-2): 101–109.
- Hinneburg, I., Dorman, H.J.D., Hiltunen, R. 2006: Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry*, 97 (1): 122–129.
- Hosseinnzadeh, H., Karimi, G.R., Ameri M. 2002: Effects of *Anethum graveolens* L. seed extracts on experimental gastric irritation models in mice. *BMC Pharmacology*, 2 (21): 1471–2210.
- Jirovetz, L., Buchbauer, G., Stoyanova, A.S., Georgiev, E.V., Damianova, S.T. 2003: Composition, quality control, and antimicrobial activity of the essential oil of long-time stored dill (*Anethum graveolens* L.) seeds from Bulgaria. *Journal of Agricultural and Food Chemistry*, 51 (13): 3854–3857.
- Kaur, G.J., Arora, D.S. 2010: Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family *Umbelliferae* - Current status. *Journal of Medicinal Plants Research*, 4 (2): 087–094.
- Kazemi, M. 2015: Phenolic profile, antioxidant capacity and anti-inflammatory activity of *Anethum graveolens* L. essential oil. *Natural Products Research*, 29 (6): 551–553.

- Kiehlbauch, J.A., Hannett, G.E., Salfinger, M., Archinal, W., Monserrat, C., Carlin, C. 2000: Use of the National Committee for Clinical Laboratory Standards Guidelines for Disk diffusion susceptibility testing in New York State Laboratories. *Journal of Clinical Microbiology*, 38 (9): 3341–3348.
- Koppula, S., Choi, D.K. 2011: *Anethum Graveolens* Linn (*Umbelliferae*), Extract Attenuates Stress-induced Urinary Biochemical Changes and Improves Cognition in Scopolamine induced Amnesic Rats. *Tropical Journal of Pharmaceutical Research*, 10 (1): 47–54.
- Kotironta, A., Lounatma, K., Haapasola, M. 2000: Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microbes and Infection*, 2 (2): 189–198.
- Lado, C., Then, M., Varga, I., Szoke, E., Szentmihalyi, K. 2004: Antioxidant property of volatile oils determined by the ferric reducing ability. *Zeitschrift Naturforschung C*, 59 (5-6): 354–358.
- 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. 649 p.
- Lu, Li.-C., Chen, Y.-W.C., Chou, C.-C. 2003: Antibacterial and DPPH Free Radical-scavenging Activities of the Ethanol Extract of propolis Collected in Taiwan. *Journal of Food and Drug Analysis*, 11 (4): 277–282.
- 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 Signpost: 105–135, Kerala, India.
- Markov, K., Frece, J., Čvek, D., Delaš, F. 2009: *Listeria monocytogenes* i drugi kontaminanti u svježem siru i vrhnju domaće proizvodnje s područja grada Zagreba. *Mljekarstvo*, 59 (3): 225–231.
- Mendonça-Filho, R.R. 2006. Bioactive phytochemicals: new approaches in the phytosciences, In: Ahmad, I., Aqil, F., Owais M. (eds.), *Modern phytomedicine, turning medicinal plants into drugs*: 1-24, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
- Milanov, D., Lazić S., Vidić B., Petronijević J., Bugarski D., Šugeljev Z. 2010: Slime production and biofilm forming ability by *Staphylococcus aureus* bovine mastitis isolates. *Acta Veterinaria* (Beograd), 60 (2-3): 217–226.
- Mišić, D., Žižović I., Stamenić M., Ašanin, R., Ristić, M., Petrović, S.D., Skala, D. 2008: Antimicrobial activity of celery fruit isolates and SFE proces modeling. *Biochemical engineering journal*, 42 (2): 148–152.
- Naigre, R., Kalck, P., Rogues, C., Roux, I., Michel, G. 1996 : Comparison of antimicrobial properties of monoterpenes and their carbonylated products. *Planta Medica*, 62 (3): 275–277.
- Nanasombat, S., Wimuttigosol P. 2011: Antimicrobial and antioxidant activity of spice essential oils. *Food Science and Biotechnology*, 20 (1): 45–53.
- Orhan, E.I., Senol, F.Z., Ozturk, N., Celik, S.A., Pular A., Kan, Y. 2013: Phytochemical contents and enzyme inhibitory and antioxidant properties of *Anethum graveolens* L. (dill) samples cultivated under organic and conventional agricultural conditions. *Food and Chemical Toxicology*, 59: 96–103.
- Peerakam, N., Wattanathorn, J., Punjaisee, S., Buamongkol, S., Sirisa-ard, P., Chansakaow S. 2014: Chemical profiling of essential oil composition and biological evaluation of *Anethum graveolens* L. (seed) grown in Thailand. *Journal of Natural Sciences Research*, 4 (16): 34–41.
- Pharmacopoeia Jugoslavica V, 2000: Savremena administracija, Beograd (in Serbian), Vol. 1: 118.
- Pina, E.S., Coppede, J. da S., Sartoratto, A., Fachin, A.L., Bertoni, B.W., Szelei de Castro França, S. de C., Pereira, A.M. 2012: Antimicrobial activity and chemical composition of essential oils from *Aloysia polystachya* (Griseb.) Moldenke grown in Brazil. *Journal of Medicinal Plants Research*, 6(41): 5412–5416.
- Pino, J.A., Rosado, A., Goire, I., Roncal, E. 1995: Evaluation of flavor characteristic compounds in dill herb essential oil by sensory analysis and gas chromatography. *Journal of Agricultural and Food Chemistry*, 43 (5): 1307–1309.
- Rădulescu, V., Popescu, M.L. Ilieș, D.-C. 2010: Chemical composition of the volatile oil from different plant parts of *Anethum graveolens* L. (*Umbelliferae*) cultivated in Romania. *Farmacia*, 58 (5): 594–600.
- Samaržija, D., Damjanović S., Pogačić T. 2007: *Staphylococcus aureus* u siru. *Mljekarstvo*, 57 (1): 31–48.
- 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.
- Singh, G., Murya S., De Lampasona, M.P., Catalan, C. 2005: Chemical constituents, antimicrobial investigations, and antioxidative potentials of *Anethum graveolens* L. essential oil and acetone extract: Part 52. *Journal of Food Science*, 70 (4): 208–215.

- Singh, P., Prakash, A. 2008: Isolation of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* from milk products sold under market conditions at Agra region. *Acta agriculturae Slovenica*, 92 (1): 83–88.
- Sintim, H.Y., Burkhardt, A., Gawde, A., Cantrell, L.C., Astatkie, T., Obour, A.E., Zheljaskov, V.D., Schlegel, V. 2015: Hydrodistillation time affects dill seed essential oil yield, composition, and bioactivity. *Industrial Crops and Products*, 63: 190–196.
- Stanojević, L.P., Radulović, N.S., Djokić, T.M., Stanković, B.M., Ilić, D.P., Cakić, M.D., Nikolić, V.D., 2015: The yield, composition and hydrodistillation kinetics of the essential oil of dill seeds (*Anethii fructus*) obtained by different hydrodistillation techniques. *Industrial Crops and Products*, 65: 429–436.
- Stanojević, J.S., Stanojević, Lj.P., Cvetković, D.J., Danilović, B.R., 2015a: Chemical composition, antioxidant and antimicrobial activity of the turmeric essential oil (*Curcuma longa* L.). *Advanced technologies*, 4 (2): 19–25.
- Tepe, B., Donmez, E., Unlu M., Candan, F., Daferera, D., Vardar-Unlu, G., Polissiou, M., Sokmen, A. 2004: Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). *Food Chemistry*, 84 (4): 519–525.
- Tepe, B., Sokmen, M., Akpulat H.A., Daferera, D., Polissiou M., Sokmen, A. 2005: Antioxidative activity of the essential oils of *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* and *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*. *Journal of Food Engineering*, 66 (4): 447–454.
- Tucakov, J. 1997: Lečenje biljem, RAD-Beograd. Beograd (In Serbian). 717 p.