



UNIVERZITET U
Kragujevcu
AGRONOMSKI FAKULTET U
Čačku



UNIVERSITY OF
Kragujevac
FACULTY OF
AGRONOMY
Čačak

XXII SAVETOVANJE O BIOTEHNOLOGIJI

sa međunarodnim učešćem

- ZBORNİK RADOVA 2 -



Čačak, 10 - 11. Mart 2017. godine

XXII SAVETOVANJE O BIOTEHNOLOGIJI

sa međunarodnim učešćem

- Zbornik radova 2 -

ORGANIZATOR I IZDAVAČ

**Univerzitet u Kragujevcu,
Agronomski fakultet u Čačku**

Organizacioni odbor

prof. dr Gordana Šekularac, dr Pavle Mašković, dr Milun Petrović, dr Gorica Paunović, prof. dr Milomirka Madić, dipl. ing. Srđan Bošković

Programski odbor

prof. dr Leka Mandić, prof. dr Vladeta Stevović, prof. dr Dragutin Đukić, prof. dr Snežana Bogosavljević-Bošković, prof. dr Tomo Milošević, prof. dr Milica Cvijović, prof. dr Radojica Đoković, prof. dr Milomirka Madić, prof. dr Goran Dugalić, prof. dr Aleksandar Paunović, prof. dr Radoš Pavlović, prof. dr Milena Đurić, prof. dr Gordana Šekularac, prof. dr Biljana Veljković, dr Nikola Bokan, dr Drago Milošević, dr Lenka Ribić-Zelenović, dr Vladimir Kurćubić, dr Goran Marković, dr Ljiljana Bošković-Rakočević, dr Gorica Paunović, dr Milun Petrović, dr Milan Lukić, dr Slavica Vesković

Tehnički urednici

dr Milun Petrović, dipl. ing. Dušan Marković, dipl. ing. Srđan Bošković

Tiraž: 180 primeraka

Štampa

Grafička radnja štamparija Bajić, V. Ignjatovića 12, Trbušani, Čačak

SADRŽAJ

Sekcija: Prehrambena tehnologija

<i>Marija Radojković, Pavle Mašković, Saša Đurović, Vladimir Filipović, Jelena Filipović, Milena Vujanović, Milica Nićetin: TEHNOLOŠKI POTENCIJAL LEKOVITOG BILJA BALKAN</i>	479
<i>Aleksandra Cvetanović, Marija Radojković, Saša Đurović, Pavle Mašković, Zoran Zeković: INOVATIVNE TEHNOLOGIJE U EKSTRAKCIJI BIOLOŠKI VAŽNIH MOLEKULA</i>	485
<i>Monika Stojanova, Igor Ivanovski, Irena Stojkova, Marina Todor Stojanova: COMPARATIVE RESEARCH FOR THE INFLUENCE OF DRYING TECHNOLOGY ON THE CHEMICAL COMPOSITION OF CHANTERELLE (Cantharellus cibarius) AND PORCINI MUSHROOMS (Boletus edulis)</i>	491
<i>Jasmina Vitas, Radomir Malbaša, Eva Lončar, Spasenija Milanović: KARAKTERISTIKE FERMENTACIJE KOMBUHE NA EKSTRAKTU CIKORIJE</i>	501
<i>Pavle Mašković, Saša Đurović, Marija Radojković, Dragutin Đukić, Leka Mandić, Milica Zelenika, Vesna Đurović: CHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF HELIANTHUS TUBEROSUS</i>	509
<i>Milan Stanković, Nenad Zlatić: KOLIČINA FENOLNIH JEDINJENJA I ANTIOKSIDATIVNA AKTIVNOST ODABRANIH VRSTA RODA TEUCRIUM</i>	515
<i>Miloš Bjelica, Vesna Vujanović: UPOREDNA KARAKTERIZACIJA SASTAVA I NUTRITIVNE VREDNOSTI HLADNO PRESOVANOG I RAFINISANOG ULJA SEMENKI GROŽĐA</i>	521
<i>Mirjana Radovanović, Vesna Đurović, Milica Gvozdinović, Branimir Jugović, Branimir Grgur, Zorica Knežević-Jugović: UTICAJ VELIČINE ČESTICA POLIANILINA NA IMOBILIZACIJU ALFA-AMILAZE</i>	527
<i>Milan Mitić, Aleksandra Pavlović, Pavle Mašković, Jelena Mitić: OPTIMIZACIJA EKSTRAKCIJE MAKROELEMENATA IZ MATIČNJAKA METODOM ODZIVNE POVRŠINE</i>	535
<i>Pavle Mašković, Saša Đurović, Marija Radojković, Aleksandra Cvetanović, Vesna Veličković, Zoran Zeković, Nemanja Miletić: ISOLATION AND CHEMICAL PROFILE OF THYMUS SERPYLLUM L. AND LAVANDULA ANGUSTIFOLIA MILL. ESSENTIAL OILS</i>	541
<i>Pavle Mašković, Dragutin Đukić, Leka Mandić, Desimir Knežević, Milica Cvijović, Marija Radojković, Saša Đurović: QUALITY AND CHEMICAL PROFILE ASSESSMENT OF DIFFERENT TEAS IN SERBIA</i>	549
<i>Mirjana Petronijević, Pavle Mašković, Aleksandra Cvetanović, Jasmina Agbaba, Jelena Molnar Jazić, Aleksandra Tubić, Božo Dalmacija: UTICAJ DOZE OZONA I UV ZRAČENJA NA POTENCIJALNU TOKSIČNOST PODZEMNE VODE NAKON O₃/UV UNAPREĐENOG OKSIDACIONOG PROCESA</i>	555
<i>Milica Zelenika, Pavle Mašković, Leka Mandić, Zvezdana Tadić, Slavica Vesković-Moračanin, Desimir Knežević, Vesna Đurović, Dragutin Đukić: ANTIBAKTERIJSKA AKTIVNOST RAZLIČITIH EKSTRAKATA HELIANTHUS TUBEROSUS L.</i>	561

ISOLATION AND CHEMICAL PROFILE OF *THYMUS SERPYLLUM* L. AND *LAVANDULA ANGUSTIFOLIA* MILL. ESSENTIAL OILS

Pavle Mašković¹, Saša Đurović², Marija Radojković², Aleksandra Cvetanović², Vesna Veličković³, Zoran Zeković¹, Nemanja Miletić²

Abstract: Aim of this study was to isolate essential oil from two different plants, *Thymus serpyllum* L. and *Lavandula angustifolia* Mill., as well to investigate their chemical composition. Essential oil was isolated by hydrodistillation, while chemical composition was established by GC-MS analysis. Obtained results showed that much more compounds were detected in *Lavandula angustifolia* Mill. than in *Thymus serpyllum* L. essential oil. Dominant compounds in *Lavandula angustifolia* Mill. were linalool (54.24%), eucalyptol (17.97%) and endo-borneol (13.36%), while thymol (37.37%), β -bisabolene (6.98%), germacrene D (6.68%) and *trans*-caryophyllene (6.47%) dominated in *Thymus serpyllum* L. essential oil.

Key words: *Thymus serpyllum* L., *Lavandula angustifolia* Mill., Essential oil, Chemical composition, GC-MS analysis

Introduction

Thymus serpyllum L. is plant from the Thymus genus and is native to the Mediterranean region and Southern Italy (Abu-Darwish et al., 2009). This genus is known for its pharmacological properties such as spasmolytic, antiseptic, antitussive, etc. (Ewans, 1998). Thymus oil is widely used due to antioxidant activity (Kuresh and Stanley, 1999), as well as antimicrobial effect (Juliano et al., 2000; Karaman et al., 2001). *Thymus serpyllum* L. (wild thyme) is well known by its application in folk medicine thorough remedies (Nikolic et al., 2014). It has been established that plant exhibits antiseptic, analgesic, carminative, expectorant, diuretic, emmenagogue and stimulant properties and is used for mouth washes, against cold and cough (Farooqi et al., 2005).

Lavandula angustifolia Mill. (*Lamiaceae* botanical family) is well known, widely distributed aromatic herb, which is used in toiletry and perfumery industries (Hajhashemi et al. 2003). It is also known as a medicinal herb and has been used for the treatment of several gastrointestinal, nervous and rheumatic disorders (Duke, 1989; Evans, 1989; Leung and Foster, 1996). Pharmacological and biological tests have demonstrated that extracts, fractions and essential oil of this plant exhibit CNS-depressant, anti-convulsive, sedative, spasmolytic, local anesthetic, antioxidant, anti-bacterial and mast cell degranulation inhibitory effects (Leung and Foster, 1996; Kim and Cho, 1999; Hohmann et al., 1999; Lis-Balchin and Hart, 1999; Ghelardini et al., 1999).

¹University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (pavlem@kg.ac.rs)

²University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, Novi Sad, Serbia

³University of Kragujevac, Faculty of Science, Radoja Domanovića 12, Kragujevac, Serbia

Essential oils are composed of volatile and odorous compounds, which have been synthesized throughout the secondary metabolism of plants. Constituents of essential oils may be divided into two major groups: hydrocarbons (terpenes, sesquiterpenes and diterpenes) and oxygenated compounds derived from these hydrocarbons (alcohols, esters, aldehydes, ketones, phenols, etc.) (Evandri et al., 2005; Da Porto et al., 2009). Essential oils have been widely used in the food industry as flavoring agents (Mazzanti et al., 1998), while their constituents exhibit wide range of biological activities such as antioxidant, antibacterial, antifungal, etc. (Lu et al., 2002; D'Auria et al., 2001).

Aim of this research was to isolate and investigate chemical composition of *Thymus serpyllum* L. and *Lavandula angustifolia* Mill. essential oils. In order to isolate essential oils, hydrodistillation process was applied, while chemical composition was established applying GC-MS analytical technique.

Material and methods

Plant material

Thymus serpyllum L. and *Lavandula angustifolia* Mill. were collected in Čačak area (Republic of Serbia) in 2016. Collected material was dried naturally in the shade on draft for one month. Dried plants were grounded in the blender and kept in the paper bags prior the usage.

Extraction procedure

Isolation of essential oil from the plant material was performed by hydrodistillation according to the standard procedure described by Ph. Jug. IV (1989).

GC-MS analysis

GC-MS analysis of essential oils was performed using Agilent Technologies GC-6890N and MS5913 mass selective detector with autosampler. System was equipped with split/splitless injector and a fused silica ZB-F MSI Phenomenex (7HG-G002-11) capillary column (30 m x 0.25 mm). The oven temperature program was as follows: from 50 °C (4 min) to 310 °C, with the step of 8 °C/min and hold 10 min. Total run time was 46 min. Injector temperature was 250 °C, pressure 9.89 psi, carrier gas was He (1.0 mL/min), split time 1 min, solvent delay 6 min, injection volume 2.0 µL. Compounds were detected by comparing the mass spectra and retention time with the data from the Willey and NIST 05 spectral libraries.

Results and discussion

Chemical profile of obtained essential oils are presented in Table 1. From the presented results, it might be noticed that far more compounds were detected in the *Lavandula angustifolia* essential oil. Presented results demonstrated significant diversity in chemical composition of essential oil isolated from these plants. Dominant

compounds in *Lavandula angustifolia* Mill. were linalool (54.24%), eucalyptol (17.97%) and endo-borneol (13.36%). On the other hand, thymol (37.37%), β -bisabolene (6.98%), germacrene D (6.68%) and *trans*-caryophyllene (6.47%) were the most abundant compounds in *Thymus serpyllum* L. essential oil.

Tabela 1. Hemisjki profil etarskih ulja lavande i majčine dušice
 Table 1. Chemical profile of obtained essential oils

Jedinjenje <i>Compound</i>	RT (min)	Sadržaj jedinjenja (%) <i>Content (%)</i>	
		<i>Lavandula angustifolia</i>	<i>Thymus serpyllum</i>
α -Pinen <i>α-Pinene</i>	7,696	0.18	ND
Kamfen <i>Camphene</i>	8.061	0.12	ND
β -Terpinen <i>β-Terpinene</i>	8.690	0.04	ND
β -Pinen <i>β-Pinene</i>	8.764	0.29	ND
1-Okten-3-ol <i>1-Octen-3-ol</i>	9.010	0.07	ND
γ -Terpinen <i>γ-Terpinene</i>	9.564	0.17	ND
Eukaliptol <i>Eucalyptol</i>	10.239	17.97	ND
β - <i>trans</i> -Ocimen <i>β-trans-Ocimene</i>	10.290	1.29	ND
α -Terpinolen <i>α-Terpinolene</i>	11.336	0.12	ND
Linalool <i>Linalool</i>	11.988	54.24	ND
Artemisia trien <i>Artemisia triene</i>	12.376	0.16	ND
Kamfor <i>Camphor</i>	12.811	4.83	0.35
endo-Borneol <i>endo-Borneol</i>	13.279	13.36	0.92
Terpinen-4-ol <i>Terpinene-4-ol</i>	13.491	2.36	0.49
α -Terpineol <i>α-Terpineol</i>	13.405	ND	0.36
Krypton <i>Crypton</i>	13.577	0.27	ND
Karvakrol metil-etar <i>Carvacrol methyl ether</i>	14.194	ND	4.34

Fural <i>Furale</i>	13.611	0.36	ND
Kamfen <i>Camphene</i>	13.674	0.72	ND
Mirtenol <i>Myrtenol</i>	13.737	0.15	ND
4-butilfenol <i>4-butyl phenol</i>	13.952	0.07	ND
<i>trans</i> -Karveol <i>trans-Carveol</i>	14.062	0.07	ND
α -Terpinen <i>α-Terpinene</i>	14,194	0.06	2.62
δ -4-Karen <i>δ-4-Carene</i>	14.240	0.14	ND
Timil metil-etar <i>Thymyl methyl ether</i>	14.371	ND	2.58
Kumin-aldehid <i>Cuminaldehyde</i>	14.462	0.33	ND
L-Karvon <i>L-Carvone</i>	14.508	0.09	ND
<i>trans</i> -Geraniol <i>trans-Geraniol</i>	14.554	ND	1.15
Linalil-acetat <i>Linalyl acetate</i>	14.651	0.77	ND
Piperiton <i>Piperitone</i>	14.691	0.03	ND
Endobornil-acetat <i>Endobornyl acetate</i>	15.160	ND	0.47
Bornilen <i>Bornylene</i>	15.217	0.28	ND
Timol <i>Thymol</i>	15.246	ND	37.37
Izotimol <i>Isotymol</i>	15.343	ND	1.11
Karvakrol <i>Carvacrol</i>	15.417	ND	0.37
Nerol <i>Nerol</i>	16.457	0.09	ND
Geranil-acetat <i>Geranyl acetate</i>	16.772	0.09	1.74
Neril-acetat <i>Neryl acetate</i>	16.783	0.18	ND
1,5,9-ciklododekatrien <i>1,5,9-cyclododecatriene</i>	16.949	ND	0.64

<i>trans</i> -Kariofilen <i>trans</i> - <i>Caryophyllene</i>	17.549	0.07	6.47
6-(1 <i>Z</i> ,3-butadienil)-1,4-cikloheptadien 6-(1 <i>Z</i> ,3- <i>Butadienyl</i>)-1,4-cycloheptadiene	17.680	ND	0.26
<i>trans</i> - β -Farnezen <i>trans</i> - β - <i>Farnesene</i>	17.989	0.12	3.30
α -Farnezen α - <i>Farnesene</i>	18.086	ND	1.43
β -Kubeben β - <i>Cubebene</i>	18.218	0.26	0.83
α -Kubeben α - <i>Cubebene</i>	18.406	ND	0.33
Germakren D <i>Germacrene D</i>	18.515	0.06	6.68
Germakren B <i>Germacrene B</i>	18.755	ND	2.12
β -Bisabolen β - <i>Bisabolene</i>	18.852	ND	6.98
γ -Kadinen γ - <i>Cadinene</i>	19.006	0.04	1.17
β -Kadinen β - <i>Cadinene</i>	19.115	ND	5.17
5,6-Dimetilen-ciklookten 5,6- <i>Dimethylene-cyclooctene</i>	19.664	ND	0.33
2-Cijano-1-cikloheksen 2- <i>Cyano-1-cyclohexene</i>	19.955	ND	0.20
Izospantulenol <i>Isospanthulenol</i>	19.995	ND	1.08
α -Farnezen α - <i>Farnesene</i>	20.086	ND	0.94
Kariofilen oksid <i>Caryophyllene oxide</i>	20.115	0.26	ND
tau-Murolol <i>tau-Muurolol</i>	21.087	ND	4.47
Levomenol <i>Levomenol</i>	21.447	0.16	ND

ND-not detected.

Previously conducted research confirmed the presence of linalool in high amount (Da Porto et al., 2009). It was also reported high amount of linalyl acetate in essential

oil of *Lavandula angustifolia* Mill. (Da Porto et al., 2009; Evandri et al., 2005; Verma et al. 2010), where it was the dominant compound in some cases (Evandri et al., 2005; Verma et al. 2010). It should be mentioned that Hajhashemi et al. (2003) reported domination of eucalyptol (65.4%) in *Lavandula angustifolia* Mill. essential oil, followed by borneol (11.5%). Domination of thymol in *Thymus serpyllum* L. essential oil was also previously reported (Wichtl, 1994; Evans, 2000; Thompson et al., 2003, Nikolic et al., 2014). Beside these reports, there were come other studies which reported different results. Rasooli and Mirmostafa (2002) showed presence of thymol in high amount (18.73%), but identified γ -terpinene (21.90%) and p-cymene (21.12%) as main compounds. Similar results were reported by Sefidkon et al. (2004), while Raal et al. (2004) found nerolidol (24.87%) as main compound followed by caryophyllene oxide (11.29%), β -caryophyllene (7.06%), germacrene D (6.52%), myrcene (5.65%) and geranyl acetate (4.67%).

Conclusion

Hydrodistillation of *Lavandula angustifolia* Mill. and *Thymus serpyllum* L. plants materials were performed in order to isolate their essential oils. Furthermore, obtained essential oil was analyzed and presented results showed high diversity in chemical compositions. Dominant compound in *Lavandula angustifolia* Mill. was linalool, while thymol was the most abundant one in *Thymus serpyllum* L. essential oil. Based on the results from the previous studies, which showed wide diversity of biological activity of essential oils, this results may be significant for pharmaceutical industry. Its significance is even greater due to the fact that identified compounds such as linalool, camphor, eucalyptol, carvacrol, pinene, as well as many others, expressed different biological properties which were previously studied and proved. Furthermore, usage of essential oil is not restricted only to the pharmaceutical industry. For such reason, these results may be relevant for other industries such as food industry.

References

- Abu-Darwish M.S, Abu Dieyeh Z.H., Mufeed B., Al-Tawaha A.R.M., Al-Dalain S.Y.A. (2009). Trace element contents and essential oil yields from wild thyme plant (*Thymus serpyllum* L.) grown at different natural variable environments, Jordan. *Journal of Food, Agriculture & Environment*, 7 (3&4), 920-924.
- D'Auria F.D., Laino L., Strippoli V., Tecca M., Salvatore G., Battinelli L., Mazzanti G. (2001). In vitro activity of the Tea tree oil against *Candida albicans* mycelial conversion and other pathogenic fungi. *Journal of Chemotherapy*, 13 (4), 377-383.
- Da Porto C., Decorti C., Kikic I. (2009). Flavour compounds of *Lavandula angustifolia* L. to use in food manufacturing: Comparison of three different extraction methods. *Food Chemistry*, 112, 1072-1078.
- Duke J.A. (1989). *Handbook of medicinal herbs*. Boca Raton, USA: CRC Press.
- Evandri M.G., Battinelli L., Daniele C., Mastrangelo S., Bolle P., Mazzanti G. (2005). The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay. *Food and Clinical Toxicology*, 43, 1381-1387.

- Evans W.C. (1989). Trease and Evans' pharmacognosy, 13th edition. London, England: Bailliere Tindal.
- Evans W.C. (1998). Trease and Evans' pharmacognosy. London, England: Bailliere Tindal.
- Evans W.C. (2000). Trease and Evans' pharmacognosy, 15th edition. Edinburgh, Scotland: Saunders.
- Farooqi A.A., Sreeramu B.S., Srinivasappa K.N. (2005). Cultivation of spice crops. Hyderabad: Universities Press.
- Ghelardini C., Galeotti N., Salvatore G., Mazzanti G. (1999). Local anesthetic activity of the essential oil of *Lavandula angustifolia*. *Planta Medica*, 65, 700-703.
- Hajhashemi V., Ghannadi A., Sharif B. (2003). Anti-inflammatory and analgesic properties of leaf extracts and essential oil of *Lavandula angustifolia* Mill. *Journal of Ethnopharmacology*, 89, 67-71.
- Hohmann J., Zupko I., Redei D., Csanyi M., Falkay G., Mathe I. (1999). Protective effect of the aerial parts of *Salvia officinalis*, *Melissa officinalis* and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. *Planta Medica*, 65, 576-578.
- Juliano C., Mattana A., Usai M. (2000). Composition and *in vitro* antimicrobial activity of the essential oil of *Thymus herba-barona* Loisel growing wild in Sardinia. *Journal of Essential Oil Research*, 12 (4), 516-522.
- Karaman S., Digrak M., Ravid U., Ilcim A. (2001). Antibacterial and antifungal activity of the essential oils of *Thymus revolutus* Celak from Turkey. *Journal of Ethnopharmacology*, 76 (2), 183-186.
- Kim H.M., Cho S.H. (1999). Lavender oil inhibits immediate-type allergic reaction in mice and rats. *The Journal of Pharmacy and Pharmacology*, 51, 221-226.
- Leung A.Y., Foster S. (1996). *Encyclopedia of common natural ingredients used in food, drugs and cosmetics*. New York, USA: Wiley.
- Lis-Balchin M., Hart S. (1999). Studies on the mode of action of the essential oil lavender (*Lavandula angustifolia* P. Miller). *Phytotherapy Research*, 13, 540-542.
- Lu M., Battinelli L., Daniele C., Malchioni C., Salvatore G., Mazzanti G. (2002). Muscle relaxing activity of *Hyssopus officinalis* essential oil on isolated intestinal preparations. *Planta Medica*, 68 (3), 213-216.
- Nikolic M., Glamoclija J., Ferreira I.C.F.R., Calhelha R.C., Fernandes A., Markovic T., Markovic D., Giweli A., Sokovic M. (2014). Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. *Industrial Crops and Products*, 52, 183-190.
- Ph. Yug. IV.* (1984). Beograd: Savezni zavod za zastitu zdravlja.
- Raal A., Paaver U., Arak E., Orav A. (2004). Content and composition of the essential oil of *Thymus serpyllum* L. growing wild in Estonia. *Medicina (Kauunas)*, 40 (8), 795-800.
- Rasooli I., Mirmostafa S.A. (2002). Antibacterial properties of *Thymus pubecens* and *Thymus serpyllum* essential oils. *Fitoterapia*, 73, 244-250.
- Sefidkon F., Dabiri M., Mirmostafa S.A. (2004). The composition of *Thymus serpyllum* L. oil. *Journal of Essential Oil Research*, 16 (3), 184-185.

- Thompson J.D., Chalchat J.C., Michet A., Linhart Y.B., Ehlers B. (2003). Qualitative and quantitative variations in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *Journal of Chemical Ecology*, 29 (4), 859-880.
- Verma R.S., Rahman L.U., Chanotiya C.S., Verma R.K., Chauhan A., Yadav A., Singh A., Yadav A.K. (2010). Essential oil composition of *Lavandula angustifolia* Mill. cultivated in the mid of Uttarakhand, India. *Journal of the Serbian Chemical Society*, 75 (3), 343-348.
- Wichtl M. (1994). Herbal drugs and phytopharmaceuticals. Stuttgart, Germany: Medpharm Scientific Publishers.
- Youdim K.A, Deans S.G. (1999). Dietary supplementation of thyme (*Thymus vulgaris* L.) essential oil during the lifetime of the rat: its effects on the antioxidant status in liver, kidney and hearth tissues. *Mechanisms of Ageing and Development*, 109 (3), 163-175.