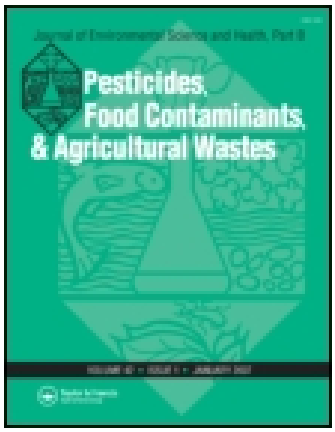
This article was downloaded by: [York University Libraries]
On: 12 August 2014, At: 10:15
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lesb20

Antibacterial activity against Clostridium genus and antiradical activity of the essential oils from different origin

Miroslava Kačániová[a], Nenad Vukovič[b], Elena Horská[c], Ivan šalamon[d], Alica Bobková[e], Lukáš Hleba[a], Martin Mellen[e], Alexander Vatľák[a], Jana Petrová[a] & Marek Bobko[f]

[a] Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

[b] Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia

[c] Department of Marketing, Faculty of Economy, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

[d] Excellence Centre of Animal and Human Ecology, Presov University in Presov, Presov, Slovakia

[e] Department of Hygiene and Food Safety, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia

[f] Department of Animal Products Evaluation and Processing, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitria, Nitra, Slovak Republic

Published online: 09 May 2014.

To cite this article: Miroslava Kačániová, Nenad Vukovič, Elena Horská, Ivan šalamon, Alica Bobková, Lukáš Hleba, Martin Mellen, Alexander Vatľák, Jana Petrová & Marek Bobko (2014) Antibacterial activity against Clostridium genus and antiradical activity of the essential oils from different origin, Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 49:7, 505-512, DOI: 10.1080/03601234.2014.896673

To link to this article: http://dx.doi.org/10.1080/03601234.2014.896673

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Journal of Environmental Science and Health, Part B (2014) **49**, 505–512
Copyright © Taylor & Francis Group, LLC
ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2014.896673

Taylor & Francis
Taylor & Francis Group

Antibacterial activity against *Clostridium* genus and antiradical activity of the essential oils from different origin

MIROSLAVA KAČÁNIOVÁ[1], NENAD VUKOVIČ[2], ELENA HORSKÁ[3], IVAN ŠALAMON[4], ALICA BOBKOVÁ[5], LUKÁŠ HLEBA[1], MARTIN MELLEN[5], ALEXANDER VATĽÁK[1], JANA PETROVÁ[1] and MAREK BOBKO[6]

[1]*Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*
[2]*Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia*
[3]*Department of Marketing, Faculty of Economy, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*
[4]*Excellence Centre of Animal and Human Ecology, Presov University in Presov, Presov, Slovakia*
[5]*Department of Hygiene and Food Safety, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia*
[6]*Department of Animal Products Evaluation and Processing, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitria, Nitra, Slovak Republic*

Downloaded by [York University Libraries] at 10:15 12 August 2014

In the present study, the antimicrobial and antiradical activities of 15 essential oils were investigated. The antimicrobial activities were determined by using agar disc diffusion and broth microdilution methods against *Clostridium* genus and antioxidant properties of essential oils by testing their scavenging effect on DPPH radicals activities. We determined the antibacterial activity of *Clostridium butyricum, Clostridium hystoliticum, Clostridium intestinale, Clostridium perfringens* and *Clostridium ramosum*. We obtained the original commercial essential oils samples of *Lavandula angustifolia*, *Carum carvi*, *Pinus montana*, *Mentha piperita*, *Foeniculum vulgare* Mill., *Pinus sylvestris*, *Satureia montana*, *Origanum vulgare* L. (2 samples), *Pimpinella anisum*, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Abies alba* Mill., *Chamomilla recutita* L. *Rausch* and *Thymus vulgaris* L. produced in Slovakia (Calendula a.s., Nova Lubovna, Slovakia). The results of the disk diffusion method showed very high essential oils activity against all tested strains of microorganisms. The best antimicrobial activity against *C. butyricum* was found at *Pimpinella anisum*, against *C. hystoliticum* was found at *Pinus sylvestris*, against *C. intestinale* was found at *Satureia hortensis* L., against *C. perfringens* was found at *Origanum vulgare* L. and against *C. ramosum* was found at *Pinus sylvestris.* The results of broth microdilution assay showed that none of the essential oils was active against *C. hystoliticum*. The best antimicrobial activity against *C. butyricum* was found at *Abies alba* Mill., against *C. intestinale* was found at *Abies alba* Mill., against *C. perfringens* was found at *Satureia montana* and against *C. ramosum* was found at *Abius alba* and *Carum carvi.* Antioxidant DPPH radical scavenging activity was determined at several solutions of oil samples (50 $\mu L.mL^{-1}$–0.39 $\mu L.mL^{-1}$) and the best scavenging effect for the highest concentration (50 $\mu L.mL^{-1}$) was observed. The antioxidant properties were different in particular plant species. The highest% of inhibition after 30 min. of reaction was observed at *Origanum vulgare* (93%), *Satureia montana* (90.66%) and *Lavandula augustifolia* (90.22%).

Keywords: Essential oils, antibacterial activity, clostridia, in vitro, antiradical activity.

Introduction

Many naturally occurring compounds present in plants, herbs, and spices have been shown to possess antimicrobial effect against food-borne pathogens. In recent years, there has been a considerable pressure from consumers to reduce or eliminate chemically synthesized additives in their foods. Most plants produce antimicrobial secondary metabolites, either as part of their normal program of growth and development or in response to pathogens attack or stress. The use of essential oils (EO) is a novel way to reduce the proliferation of microorganisms. The oils are natural products extracted from plant materials. They can be used as natural additives in many foods because of their antibacterial, antifungal, antioxidant and anticarcinogenic properties.[1–3] EOs have been proven to be inhibitors of a wide range of food spoiling microbes, depending on their concentration, testing methods and the presence of active constituents.[4]

Address correspondence to Miroslava Kačániová, Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovak Republic; E-mail: miroslava.kacaniova@gmail.comReceived August 30, 2013.

The antimicrobial compounds found in plants are interesting because the antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections.[5,6]

In the present study, the antibacterial and antioxidant capacities of the 15 EOs were investigated. The antimicrobial activities were determined by using agar disc diffusion and broth microdilution methods against *Clostridium* genus. The antioxidant activities were determined as inhibition of DPPH radical.

Material and methods

Essential oil samples

We used the original EOs of *Lavandula angustifolia*, *Carum carvi*, *Pinus montana*, *Mentha piperita*, *Foeniculum vulgare* Mill., *Pinus sylvestris*, *Satureia montana*, *Origanum vulgare* L. (2 samples), *Pimpinella anisum*, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Abies alba* Mill., *Chamomilla recutita* L. *Rausch* and *Thymus vulgaris* L. All these samples were produced in Slovakia (Calendula a.s., Nova Lubovna). The samples were stored in the dark at the temperature of 4°C.

Preparing essential oil samples and chemical composition

The medicinal plants for EO isolation were donated by successful and established growers. EOs were distilled in the large-scale distillation apparatus specifically designed for aromatic and medicinal plants. There are two types: Type HV-3000 (height: 5250 mm, width: 2180 mm, with container for 200 or 250 kg of dried matter of 400 or 500 kg of fresh matter of plant material) and Type HV-300 (height: 3400 mm, width: 1300 mm, with container for 40 or 50 kg of dry matter and 100 or 120 kg of fresh matter of plant material). This large-scale technology of EO distillation in this Slovak company consists from the main distillatory apparatus, a steam condenser and additional apparatuses (steam boiler and apparatus for improving of used water). Analysis of the EOs was carried out using a Hewlett-Packard 5890/5970 GC-MSD system (DSB Scientific, New Bern, NC, USA).

Chemical composition of EOs were as follows: *Lavandula angustifolia*: linalyl acetate (46%), linalool (26%), lavandulyl acetate (3%) and α-terpineol (4%); *Carum carvi*: carvone (70%), limomene (27%); *Pinus montana*: α-pinene (24%), β-pinene (7%), α-phellandrene (8%), bornyl acetate (8%), camphene (13%) and limonene (4%); *Mentha piperita*: menthol (46%), menthofuran (22.6%), (-)-metylacetate (3.5%), neomenthol (3.6%), pulegone (1.9%), isomenthone (8.8%) and linalool (0.6%); *Foeniculum vulgare* Mill.: t-anethol (33%); *Pinus sylvestris:* α-pinene (27.4%), β-pinene (9.7%), α-phellandrene (10.0%), bornyl acetate (18.8%), camphene (15.5%) and limonene (6.1%); *Satureia montana*: carvacrol (45%), γ-terpinene (30%), α-terpinene (3%), p-cimene (2%); *Origanum vulgare* L.: carvacrol (55%); *Pimpinella anisum* anethol (80%); *Rosmarinus officinalis* L.: α-pinene (11.9%), camphene (8.4%), β-pinene (2.8%), limonene (11.5%), 1,8-cineole (34.4%), camphor (7.4%), borneol (4.4%), α-terpineol (3.3%) and boranyl acetate (4.4%); *Salvia officinalis* L.: α-pinene (9.2%), 1,8-cineole (12..6%), α-thujone (24.7%), β-thujone (5.2%), camphor (16.8%), β-caryophyllene (5%) and α-caryophyllene (2.8%); *Abies alba* Mill.: bornyl acetatte (30%), camphene (18%), α-pinene (3%), borneol (1.5%), α-terpinene (1.2%); *Chamomilla recutita* L. *Rausch:* /-/-α-bisabolol (34.2%), chamazulene (11.3%), trans-β-farnesene (35.7%) and *Thymus vulgaris* L.: p-cimen (22%), thymol (43%) and carvacrol (2%).

Antimicrobial activity

Antibacterial activity was assessed on *Clostridium butyricum*, *Clostridium hystoliticum*, *Clostridium intestinale*, *Clostridium perfringens* and *Clostridium ramosum* assessed. These samples came from the collections of the Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovakia. The mother cultures of each clostridium were set up 24 h before the assays in order to reach the stationary phase of growth. The tests were assessed by inoculating Petri dishes from the mother cultures with proper sterile media. The main aim was to obtain the microorganism concentration of 10^5 colony forming units cfu mL^{-1}.

Disc diffusion method

We used the agar disc diffusion method for the determination of antimicrobial activities of the EO.[7] Briefly, a suspension of the tested microorganism (0.1 mL of 10^5 cells per mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 μL of the oil and placed on the inoculated plates. They were inoculated onto the surface of Mueller Hinton Agar (MHA, Oxoid, Basingstoke, UK). These plates, after remaining at 4°C for 2 h, were incubated anaerobically at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in duplicate.

Determination of minimum inhibitory concentration

Broth microdilution susceptibility assay was used, as recommended by NCCLS, for the determination of the MIC.[8] All tests were performed in Mueller Hinton Broth (MHB; Oxoid) supplemented with Tween 80 detergent (final concentration of 0.5% $v.w^{-1}$). Bacterial strains were cultured overnight at 37°C in MHA. Test strains were suspended in MHB to give a final density of 5.10^5 cfu.mL^{-1} and these were confirmed by viable counts. The test oil

Downloaded by [York University Libraries] at 10:15 12 August 2014

solution was prepared in dimethyl sulfoxide (DMSO, Penta, Prague, Czech Republic). Geometric dilutions, ranging from 0.0235 to 0.75 $\mu L\ mL^{-1}$ of the EO, were prepared in a 96-well microtitre plate, including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). The plates were incubated under anaerobic conditions, at 37°C for 24 h for bacteria. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

DPPH radical scavenging activity

Free radical scavenging activities of the EOs were evaluated in accordance with method of Takao et al.[9] with some modification. 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Bratislava, Slovakia) (8 mg) was dissolved in absolute methanol (100 mL) to obtain a concentration of 80 $mg.mL^{-1}$. Diluted solutions of oil samples (50 $\mu L.mL^{-1}$–0.39 $\mu L.mL^{-1}$) were mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur. Absorbance was recorded at 517 nm using T80 UV/Vis Double Beam Spectrophotometer (Oasis Scientific, Greenville, SC, USA). Antioxidant activity was expressed as percentage (%) of scavenging activity:

$$\%\ \text{inhib.} = [(\text{ADPPH} - \text{Asample})/\text{ADPPH}] \times 100$$

Statistical analysis

The basic variation–statistical values from obtained data were calculated by using statistical program Statgraphic (Statpoint technologies, Warrenton, VA, USA). In this study, following values were calculated: average, standard deviation, minimum, and maximum, coefficient of variation and frequency of size of inhibition zones.

Results and discussion

Antibacterial activity

Aromatic plants are frequently used in traditional medicine as the antimicrobial agents and their EOs, mixtures of natural volatile compounds isolated by steam distillation, have been known since antiquity to possess antibacterial and antifungal properties.[10] The diffusion method is generally used as a preliminary screening for antimicrobial activity prior to more detailed studies.[11] Usefulness of this method is limited to the generation of preliminary quantitative data only, as the hydrophobic nature of most EOs and plant extracts components prevents their uniform diffusion through the agar medium. Based on this, it is recommended to use an emulsifier such as DMSO, to assure contact between the microorganism and the possible antimicrobial agent.[12]

The biological activity against clostridia was determined by employing the standard discs diffusion technique (Table 1). The results from the disk diffusion method assays showed variable activity against all tested strains of clostridia. *Clostridium butyricum* showed higher susceptibility in all tested EOs (6.35 mm). Antibacterial activity of EOs against *C. butyricum* ranged from 3.0 ± 0.82 at *Carum carvi* to 8.3 ± 0.96 mm at *Pimpinella anisum*. Disc diffusion method showed that antibacterial activity against *C. hystoliticum* ranged from 1.25 ± 0.50 at *Chamomilla recutita* L. to 7.75 ± 0.96 mm at *Pinus sylvestris*, against *C. intestinale* ranged from 2.00 ± 0.82 at *Origanum vulgare* to 12.25 ± 2.22 mm at *Satureia montana*, against *C. perfringens* ranged from 1.00 ± 0.00 at *Abius alba* and *Chamomilla recutita* L. to 6.50 ± 3.70 mm at *Origanum vulgare* L. and against *C. ramosum* from 1.00 ± 0.00 *Carum carvi*, *Pimpinella anisum* and *Rosmarinus officinalis* L. to 7.50 ± 0.58 mm at *Abies alba* Mill. The best

Table 1. Antimicrobial activity of the 15 essential oils against clostridia using agar disc diffusion in mm.

Essential oil	C. butyricum	C. hystoliticum	C. intestinale	C. perfringens	C. ramosum
Lavandula angustifolia	6.50 ± 1.29	5.25 ± 4.03	4.00 ± 1.81	3.50 ± 0.58	3.25 ± 0.96
Carum carvi	3.00 ± 0.82	2.75 ± 2.06	3.00 ± 1.83	2.25 ± 1.50	1.00 ± 0.00
Pinus Montana	7.50 ± 0.58	4.25 ± 2.22	5.50 ± 2.52	1.00 ± 0.00	7.50 ± 0.58
Mentha piperita	5.00 ± 0.82	3.25 ± 0.50	3.50 ± 0.58	3.00 ± 0.82	4.25 ± 0.96
Foeniculum vulgare Mill.	7.50 ± 0.58	1.25 ± 0.50	6.50 ± 1.29	1.00 ± 0.00	6.50 ± 1.29
Pinus sylvestris	6.00 ± 0.82	7.75 ± 0.96	6.75 ± 1.71	5.00 ± 0.82	5.00 ± 0.82
Satureia montana	6.80 ± 1.26	4.25 ± 1.25	12.25 ± 2.22	6.00 ± 0.82	4.00 ± 0.82
Origanum vulgare L.	8.00 ± 0.00	1.50 ± 0.58	2.00 ± 0.82	6.50 ± 3.70	3.50 ± 1.29
Pimpinella anisum	8.30 ± 0.96	3.75 ± 1.50	5.50 ± 0.58	5.00 ± 0.82	1.00 ± 0.00
Rosmarinus officinalis L.	5.00 ± 0.82	4.25 ± 0.96	5.50 ± 1.73	2.00 ± 0.00	1.00 ± 0.00
Salvia officinalis L.	2.25 ± 0.5	1.25 ± 0.50	3.5 ± 0.58	3.75 ± 0.96	1.00 ± 0.00
Abies alba Mill.	7.00 ± 2.16	3.00 ± 0.82	4.50 ± 1.29	9.75 ± 2.22	3.50 ± 1.29
Chamomilla recutita L. *Rausch*	1.75 ± 0.96	3.75 ± 0.96	4.25 ± 0.96	6.00 ± 1.83	1.75 ± 0.50
Thymus vulgaris	3.75 ± 0.96	8.75 ± 0.96	10.50 ± 1.29	2.25 ± 1.50	3.75 ± 2.06
Origanum vulgare L.	2.50 ± 0.58	1.25 ± 0.50	4.00 ± 0.82	2.00 ± 0.82	1.25 ± 0.50

Downloaded by [York University Libraries] at 10:15 12 August 2014

Downloaded by [York University Libraries] at 10:15 12 August 2014

antibacterial activity against all tested clostridia was found at *Satureia hortensis*.

Minimum inhibitory amounts (MIC) determined by dilution method using Mueller Hinton broth are summarized in Table 2. The lowest minimum inhibitory effect against tested clostridia was found at *Abies alba* Mill. Minimal inhibition concentration against *C. butyricum* ranged from 0.38 at *Abies alba* Mill. to 34.03 μL mL^{-1} at *Rosmarinus officinalis* L., against *C. intestinale* ranged from 3.00 at *Abius alba* to >76.00 μL mL^{-1} at three EOs (*Lavandula angustifolia, Satureia hortensis, Pimpinella anisum*), against *C. perfringens* from 1.79 at *Satureia hortensis L.* to >76.00 μL mL^{-1} at *Pimpinella anisum* and against *C. ramosum* from 0.50 at *Abies alba* Mill. and *Carum car*vi to 22.45 μL mL^{-1} at *Chamomilla recutita* L. No antibacterial activity against *C. hystoliticum* was found.

The antimicrobial properties of winter savory EO are related to the presence of their major chemical compounds, such as thymol and carvacrol in the EO fraction.[13,14] The formation of growth inhibition zones on the tested growth bacterial cultures showed the antimicrobial effect of *Satureia montana* EO. The MIC is cited by most researchers as the measure of performance of antibacterial Eos.[15] Several authors have reported the antimicrobial effect of *S. montana* EO *in vitro*. Mirjana and Nada [13] observed the antimicrobial activity of savory EO on Gram-negative and Gram-positive bacteria, filamentous fungi and yeasts using the agar dilution method. Bezbradica et al.[16] found that *S. montana* EO in a 5% ethanol solution has wide antimicrobial activities against several microorganisms using the same methodology as we used in this study. Ćavar et al.[17] reported the antimicrobial effect of *S. montana* EO obtained by hydrodistillation using the disc diffusion method. Si et al.[18] studied the inhibition potential of 66 EOs and several of their components on *C. perfringens* type A, and they found an inhibition of over 80% in 33 of the tested components. The reported MIC values ranged between 167 and 425 μg mL^{-1}, with thymol and carvacrol as the most efficient inhibitors among the tested by the authors. The MIC values for *S. montana* EO against *C. perfringens* were not reported, so further comparisons were not made. In our study the best antimicrobial activity of EOs was found against *C. intestinale,* using disc diffusion method while

Table 2. Antimicrobial activity expressed as minimum inhibitory concentration (MIC) against clostridia strains of 15 essential oils.

Essential oil	*MIC*	C. ramnosum	C. butyricum	C. perfringens	C. intestinale
Lavandula angustifolia	50	1.70	NA	6.81	>76
	90	2.68	14.09	10.59	>76
Carum carvi	50	0.50	2.00	2.66	11.01
	90	0.56	2.24	4.64	18.23
Pinus Montana	50	19.00	34.03	4.00	>76
	90	21.60	56.41	4.47	>76
Mentha piperita	50	0.50	4.00	2.00	13.45
	90	0.56	4.47	2.24	14.47
Foeniculum vulgare Mill.	50	22.45	11.82	34.03	9.42
	90	44.78	20.25	56.41	22.32
Pinus sylvestris	50	4.69	3.00	5.33	6.00
	90	7.39	3.20	9.18	6.38
Satureia montana	50	6.00	20.28	1.79	3.00
	90	6.38	51.13	3.79	3.20
Origanum vulgare L.	50	6.13	NA	2.12	5.40
	90	10.83	1.80	3.93	10.55
Pimpinella anisum	50	22.14	9.49	>76	>76
	90	43.79	NA	>76	>76
Rosmarinus officinalis L.	50	3.00	0.38	11.91	8.00
	90	3.20	0.40	20.48	8.93
Salvia officinalis L.	50	1.50	0.38	4.00	5.41
	90	1.60	0.40	4.47	9.41
Abies alba Mill.	50	0.25	0.25	2.00	1.70
	90	0.28	0.28	2.24	2.68
Chamomilla recutita L. *Rausch*	50	8.02	33.20	NA	1.86
	90	21.59	>76	NA	3.17
Thymus vulgaris	50	7.59	NA	>76	11.82
	90	14.58	NA	>76	20.25
Origanum vulgare L.	50	1.29	8.26	27.22	2.51
	90	4.03	NA	>76	4.21

[a]MIC was considered as the lowest concentration of each essential oil; NA = no antimicrobial effect.

Downloaded by [York University Libraries] at 10:15 12 August 2014

broth diffusion method showed *C. perfringens* to be the most sensitive clostridium. Candan et al.[19] studied the antimicrobial effect of *Achillea millefolium* subsp. *millefolium* Afan against different bacteria and they observed that oil showed antimicrobial activity against *Streptococcus pneumoniae, Clostridium perfringens*, *Candida albicans*, *Mycobacterium smegmatis, Acinetobacter lwoffii* and *Candida krusei* while water-insoluble parts of the methanolic extracts exhibited slight or no activity. This study confirms that the EO of *Achillea millefolium* possesses antimicrobial properties *in vitro*.

The antimicrobial activity of the hexane fraction of rosemary extract against several Gram positive bacteria was reported by Campo and Amiot [20] with MIC ranging from 0.06% to 1.0%. Antimicrobial activity of edible coatings enriched with rosemary were studied in vitro and in vivo, which offered a great advantage in the prevention of bacterial population and browning reactions which typically result in quality loss in fruits and vegetables.[21] Using disc diffusion method, the best antibacterial activity was found against *C. intestinale* and *C. ramosus* minimal inhibition concentration was 3.00 μL mL^{-1} in our study.

The study of Cui et al.[22] showed that 15 of 90 tested plants (16.7%) exhibited complete inhibition (++) of the *Clostridium* spp. below 0.5% level of addition. Dorman and Deans [23] reported that thymol has greater inhibitory activity against Gram-positive organisms such as *Bacillus subtilis* and *Clostridium sporogenes.* We found the best antibacterial activity of mentha EO against *C. bytiricum* (5.0 $\pm$ 0.82 mm) using the disc diffusion method while broth diffusion method showed *C. perfringens* to be the most sensitive bacteria. Antimicrobial activities of *Mentha piperita* essential oil (MPEO) have been studied previously and showed that the EO could inhibit the growth of different Gram-positive and Gram-negative bacteria. Menthol as a major constituent in MPEO was shown to be an active component against *C. sporogenes. Mentha arvensis* var. *piperacens* has shown an effective antimicrobial inhibition against *C. perfringens* determined by diameter of inhibition zone. The MIC value of *Satureja montana* L. EO against *C. perfringens* was reported to be 1.56% which was higher than the value obtained for MPEO (0.05%) and showed lower antibacterial activity.[24]

The antibacterial activity of EOs from 10 aromatic plants *Matricaria chamommilla*, *Mentha piperita*, *M. spicata*, *Lavandula angustifolia*, *Ocimum basilicum*, *Thymus vulgaris*, *Origanum vulgare*, *Salvia officinalis*, *Citrus limon* and *C. aurantium* have been determined in the study of Soković et al.[25] They found that the highest and broadest activity was shown by *Origanum vulgare* oil.

Our results with *Carum carvi* EO showed that the most sensitive bacteria using disc diffusion method were *C. butyricum* and *C. intestinale*, while using broth method it was *C. perfringens*. Antibacterial activity of *Carum carvi*, determined using agar diffusion method, was observed against Gram-positive and Gram-negative bacterial species in this study.[26] The activity was particularly high against the genera *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Erwinia*, *Xanthomonas*, *Ralstonia* and *Agrobacterium*, which are responsible for plant or cultivated mushroom diseases worldwide. In general, a lower activity was observed against bacteria belonging to the genus *Pseudomonas*. These results suggest potential use of the above EOs for the control of bacterial diseases.[26]

Antimicrobial activity of EO of *Pinus halepensis* tree from Ghazaouet (Tlemcen) against bacteria (*Staphylococcus aureus* ATCC25 923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC25 922 et ATCC25 921, *Bacillus cereus* ATCC 11778) was determined Abi-Ayada et al.[27] who evaluated two methods, disc diffusion and broth dilution. EO of *Pinus halepensis* showed moderate activity against all tested strains except *P. aeruginosa* end *E. coli*, which were found to be very resistant.[27] In our study, the best antibacterial activity using disc diffusion method was found at *Pinus sylvestris* against *C. hystoliticum* and using broth diffusion method against *C. butyricum*.

Our study showed that antibacterial activity of lavandula EO has good antibacterial activity against *C. butyricum* using disc diffusion method and against *C. ramosum* using broth diffusion method. In the study of Hanamanthagouda et al.[28] *Lavandula bipinnata* EO was evaluated for antimicrobial activity against gram positive (*E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, VRE ATCC 51299, *B. subtilis*, *Micrococcus*), gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Shigella dysentery*) bacteria and fungi (*Aspergillus niger*, *Penicillium notatum* and *Candida albicans*). It was found to be active against all the microbes used for the activity. The EO was very active against *B. subtilis*, *S. aureus*, *Micrococcus*, *A. niger*, moderately active against *E. coli*, *Sh. dysentery*, *E. feacalis*, VRE, *C. albicans* and it showed low activity against *P. aureginosa* and *P. notatum*.

The antimicrobial test results of Sahin et al.[29] study showed that the EO of *O. vulgare* ssp. *vulgare* had great potential of antimicrobial activity against all 10 bacteria, and 15 fungi and yeast species tested. The strongest antibacterial activity, determined by microdilution method, was detected on G^+ bacteria such as *Bacillus species* and *Staphylococcus aureus*, obtaining minimal inhibitory concentration (MIC) of 0.16 mg mL^{-1} while antifungal activity was moderate with MICs between 2.5 and 20 mg mL^{-1}.[30] The best antibacterial activity obtaining MIC in our study with oregano EO was detected against *C. perfringens*.

Chamomile extracts exhibited considerable antimicrobial activity against all tested strains, particularly against Gram-positive bacteria. Results of disc diffusion method, followed by measurement of MIC, indicated that *B. cereus* and *A. flavus* were the most sensitive microorganisms tested, showing the largest inhibition zones and the lowest MIC values. Least activity was exhibited against *E. coli*,

Downloaded by [York University Libraries] at 10:15 12 August 2014

with the smallest inhibition zones and the highest MIC value.[31] Cantore et al.[32] reported that the Gram-negative strains of bacteria, especially *E. coli*, have less sensitivity to chamomile EO. In our study we found good antimicrobial activity against three of five tested clostridia, using disc diffusion method.

We found that *Pimpinella anisetum* showed best antimicrobial activity against *C. butyricum*, using both methods. Tepe et al.[33] found that *C. perfringens* is the most sensitive microorganism with the lowest MIC value (4.50 mg mL^{-1}). The results were obtained by the disc diffusion method, followed by measurements of MIC. Other sensitive microorganisms are *S. peumoniae* and *A. lwoffii* with the same MIC values (18 mg mL^{-1}). According to the results obtained from *P. flabellifolia* oil, *C. perfringens* is again the most sensitive microorganism with the lowest MIC value (2.25 mg mL^{-1}), followed by *S. peumoniae* (9.0 mg mL^{-1}). In addition to these findings, *C. albicans* and *C. krusei* (yeasts) exhibited sensitivity to both oils with an MIC value of 36 mg mL^{-1}.

Antioxidant activity

The EO and various extracts were subjected to screening for their possible antioxidant activity. Free radical scavenging capacities of the extracts, measured by DPPH assay, are shown in Table 3. As the best scavenging solutions of DPPH radical were the oils at the highest concentration 50 $\mu L.mL^{-1}$, with the decreasing concentration of oil solution antioxidant activity was reduced (not shown).

The antiradical properties were different in particular plant species. The highest% of inhibition after 30 min. of reaction was observed at *Origanum vulgare* (93.00%), *Satureia montana* (90.66%) and *Lavandula augustifolia* (90.22%).

Table 3. Antioxidant activity expressed as % inhibition of DPPH radical at 50 $\mu L\ mL^{-1}$ concentration of oil samples.

Essential oil	*Antioxidant activity (%)*
Lavandula angustifolia	90.22
Carum carvi	76.67
Pinus Montana	50.54
Mentha piperita	37.43
Foeniculum vulgare Mill.	53.52
Pinus sylvestris	82.09
Satureia montana	90.66
Origanum vulgare L.	77.48
Pimpinella anisum	58.66
Rosmarinus officinalis L.	47.15
Salvia officinalis L.	68.55
Abies alba Mill.	56.17
Chamomilla recutita L. *Rausch*	56.27
Thymus vulgaris	86.80
Origanum vulgare L.	93.00

Antiradical properties decreased in the following order: *Origanum vulgare* (93.00%) > *Satureia montana* (90.66%) > *Lavandula augustifolia* (90.22%) > *Thymus vulgaris* (86.8%) > *Pinus sylvestris* (82.09%) > *Origanum vulgare* (77.48%) > *Carum carvi* (76.67%) > *Salvia officinals* (68.55%) > *Pimpinella anisum* (58.66%) > *Chamomilla recutita* (56.27%) > *Abies alba* (56.17%) > *Foeniculum vulgare* (53.52%) > *Pinus montana* (50.54%) > *Rosmarinus officinalis* (47.15%) > *Mentha piperita* (37.43%).

DPPH radical-scavenging activities of 11 EOs were studied in the work Sacchetti et al.[34] *Rosmarinus officinalis* EO notably reduced the concentration of DPPH free radical, with an efficacy slightly lower than that of reference oil *Thymus vulgaris* (75.6 ± 0.53% inhibition). We have similar results in our study. In the study of Gulcin et al.[35] antioxidant activity of anise ethanolic extract was found to be 34.49%, which was lower than our study with anise showed (58.66%).

Antioxidant activities of the extracts were evaluated using the DPPH• radical scavenging. The analysis showed that the highest antioxidant activity was noticed for chamomile extract comparison with fennel of Roby et al. study.[31] Similar results were found in our study. The EO and different extracts of *M. piperita* were explored for antioxidant activity by evaluating their antioxidant capacity as DPPH radical scavenging activity. Peppermint oil showed almost equal antioxidant potency (about 90%).[36] *Pinus* samples in the study[37] were tested in four in vitro assays for evaluation of their antioxidant potentials at concentrations of 250, 500, and 1000 g mL^{-1}. The *Pinus* extracts exhibited weak to moderate scavenging activity against DPPH radical, which are results equal to those in our study.

Grzegorczyk et al.[38] studied the radical scavenging activity, expressed as the percentage of reduction of the initial DPPH absorbance and EC50 value, of the *Salvia officinalis* L extracts. The highest radical scavenging activity was detected for RR methanolic extract (from roots of in vitro regenerated sage plants) and HR methanolic extract (from transformed roots grown in an in vitro culture). Free radicals amount in the test sample with these extracts at the concentration of 50 $\mu g.mL^{-1}$ after 30 min incubation decreased, respectively, by 94% and 88%. Our results showed a lower antioxidant activity of sage EO (68.55%).

Conclusion

The results we obtained can be considered as the first information about antimicrobial properties of *Lavandula angustifolia*, *Carum carvi*, *Pinus montana*, *Mentha piperita*, *Foeniculum vulgare* Mill., *Pinus sylvestris*, *Satureia montana*, *Origanum vulgare* L. (2 samples), *Pimpinella anisum*, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Abies alba* Mill., *Chamomilla recutita* L. *Rausch* and *Thymus vulgaris*

L. EO samples produced in Slovakia against five different clostridia. It could contribute to knowledge about the antimicrobial potentials of these EOs against anaerobic microorganisms such as clostridia in the food. The best antimicrobial activity using disc diffusion method was found at *Satureia montana* and *Thymus vulgaris* and using broth dilution method at *Lavandula angustifolia.* In conclusion, we can consider that EOs had different antioxidant activity, with the best antioxidant activity of oregano, savory and lavender.

Funding

The paper was supported by the project grant of KEGA 013SPU-4/2012, VEGA 1/0611/14, and Food and Agriculture COST Action FA1202.

Downloaded by [York University Libraries] at 10:15 12 August 2014

References

[1] Lambert, R.J.W.; Skandamis, P.N.; Coote, P.J.; Nychas, G.J.E. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. **2001**, *91*, 453–462.

[2] Soliman, K.M.; Badeaa, RI. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol. **2002**, *40*, 1669–1675.

[3] Teissedre, P.L.; Waterhouse, A.L. 2000. Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. J. Agric. Food Chem. **2000**, *48*, 3801–3805.

[4] Smith-Palmer, A.; Stewart, J.; Fyfe, L. The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol. **2001**, *18*, 463–470.

[5] Hsueh, P.R.; Chen, W.H.; Teng, L.J.; Luh, K.T. Nosocomial infections due to methicillin- resistant *Staphyloccus aureus* and vancomycin resistant enterococci at a university hospital in Taiwan from 1991 to 2003: resistance trends, antibiotic usage and in vitro activities of new antimicrobial agents. Int. J. Antimicrob. Agents **2005**, *26*, 43–49.

[6] Mora, A.; Blanco, J.E.; Blanco, M.; Alonso, M.P.; Dhabi, G.; Echeita, A.; Gonzalez, E.A.; Bernardez, M.I.; Blanco, J. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol. **2005**, *156*, 793–806.

[7] Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk Susceptibility Test*, 7th Informational Supplement; CLSI: Wayne, PA, 1997.

[8] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*, 23rd Informational Supplement; CLSI: Wayne, PA, 1999.

[9] Takao, T.; Watanabe, N.; Yagi, I.; Sakata, K. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotechnol. Biochem. **1994**, *58*, 1780–1783.

[10] Lopes-Lutz, D.; Alviano, D.S.; Alviano, C.S.; Kolodziejczyk, P.P. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry. **2008**, *69*, 1732–1738.

[11] Hammer, K.A.; Carson, C.F.; Riley, T.V. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. **1999**, *86*, 985–990.

[12] Hili, P.; Evans, C.S.; Veness, R.G. Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Lett. Appl. Microbiol. **1997**, *24*, 269–275.

[13] Mirjana, S.; Nada, B. Chemical composition and antimicrobial variability of *Satureja montana* L. essential oils produced during ontogenesis. J. Essen. Oil Res. **2004**, *16*, 387–391.

[14] Radonic, A.; Milos, M. Chemical composition and in vitro evaluation of antioxidant Effect of free volatile compounds from *Satureja montana* L. Free Radi. Res. **2003**, *37*, 673–679.

[15] Burt, S. Essential oils: their antibacterial properties and potential applications in foods: a review. Int. J. Food Microbiol. **2004**, *94*, 223–253.

[16] Bezbradica, D.I.; Tomovic, M.J.; Vukasinovic, M.S.; Siler-Marinkovic, S. Composition and antimicrobial activity of essential oil of *Satureja montana* L. collected in Serbia and Montenegro. J. Essen. Oil Res. **2005**, *17*, 462–465.

[17] Ćavar, S.; Maksimović, M.; Šolic, M.E.; Mujkić, A.J.; Bešta R. Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. Food Chem. **2008**, *111*, 648–653.

[18] Si, W.; Ni, X.; Gong, J.; Yu, H.; Tsao, R.; Han, Y.; Chambers, J. R. Antimicrobial activity of essential oils and structurally related synthetic food additives towards *Clostridium perfringens*. J. Appl. Microbiol. **2009**, *106*, 213–220.

[19] Candan, F.; Unlu, M.; Tepe, B.; Daferera, D.; Polissiou, M.; Sökmen, A.; Akpulat, H.A. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (*Asteraceae*). J. Ethnopharmacol. **2003**, *87*, 215–220.

[20] Campo, D.J.; Amiot, J.M. The NC. Antimicrobial effect of rosemary extracts. J. Food Prot. **2000**, *63*, 1359–1368.

[21] Ponce, A.; Roura, S.; del Valle, C.; Moreira, M. Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: in vitro and in vivo studies. Postharv. Biol. Technol. **2008**, *49*, 294–300.

[22] Cui, H.; Gabriel, A.A.; Nakano, H. Antimicrobial efficacies of plant extracts and sodium nitrite against *Clostridium botulinum*. Food Contr. **2010**, *21*, 1030–1036.

[23] Dorman, H.; Deans, S. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. **2000**, *88*, 308–316.

[24] Moarefian, M.; Barzegar, M.; Sattari, M.; Naghdi Badi, H. Production of functional cooked sausage by *Mentha piperita* essential oil as a natural antioxidant and antimicrobial material. J. Med. Plants **2012**, *11*, 46–57.

[25] Soković, M.; Marin, P.D.; Brkić, D.; van Griensven, L.J.L.D. Chemical composition and antibacterial activity of essential oils of ten aromatic plants against human pathogenic bacteria. J. Agricul. Food Chem. **2005**, *53*, 57–61.

[26] Iacobellis, N.S.; Lo Cantore, P.; Capasso, F.; Senatore, F. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J. Agric. Food Chem. **2005**, *53*, 57–61.

[27] Abi-Ayada, M.; Abi-Ayada, F.Z.; Lazzounia, H.A.; Rebiahi, S.A. Antibacterial activity of *Pinus halepensis* essential oil from Algeria (Tlemcen). J. Nat. Prod. Plant Resour. **2011**, *1*, 33–36.

[28] Hanamanthagouda, M.S.; Kakkalameli, S.B.; Naik, P.M.; Nagella, P., Seetharamareddy, H.R.; Murthy, H.N. Essential oils of *Lavandula bipinnata* and their antimicrobial activities. Food Chem. **2010**, *118*, 836–839.

[29] Şahin, F.; Güllüce, M.; Daferera, D.; Sökmen, A.; Sökmen, M.; Polissiou, M.; Agar, G.; Özer, H. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. Food Contr. **2004**, *15*, 549–557.

[30] Licina, B.Z.; Stefanovi, O.D.; Vasi, S.M.; Radojevi, I.D.; Deki, M. S.; Comi, L.R. Biological activities of the extracts from wild growing *Origanum vulgare* L. Food Contr. **2013**, *33*, 498–504.

[31] Roby, M.H.H.; Sarhana, M.A.; Selim, K.H.A.; Khalel, K.I. Antioxidant and antimicrobial activities of essential oil and extracts of

fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). Industr. Crops Prod. **2013**, *44*, 437–445.

[32] Cantore, P.L.; Iacobellis, N.S.; Marco, A.D.; Capasso, F.; Senatore, F. Antibacterial activity of *Coriandrum sativum* L. and *Foeniculum vulgare* miller Var. *Vulgare* (Miller) essential oils. J. Agric. Food Chem. **2004**, *52*, 7862–7866.

[33] Tepe, B.; Akpulat, H.A.; Sokmen, M.; Daferera, D.; Yumrutas, O.; Aydin, E.; Polissiou, M.; Sokmen, A. Screening of the antioxidative and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. Food Chem. **2006**, *97*, 719–724.

[34] Sacchetti, G.; Maietti, S.; Muzzoli, M.; Scaglianti, M.; Manfredini, S.; Radice, M.; Bruni, R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. **2005**, *91*, 621–632.

[35] Gulcin, I.; Oktay, M.; Kırecci, E.; Kufreviaglu, O.I. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food Chem. **2003**, *83*, 371–382.

[36] Singh, R.; Shushni, M.A.M.; Belkheir, M. Antibacterial and antioxidant activities of *Mentha piperita* L. Arab. J. Chem. in press. Available online at http://www.sciencedirect.com/science/article/pii/S1878535211000232 (accessed 2011).

[37] Ustun, O.; Senol, F.S.; Kurkcuoglu, M.; Orhan, I.O.; Kartal, M.; Can Baser, K.H. Investigation on chemical composition, anticholinesterase and antioxidant activities of extracts and essential oils of Turkish *Pinus* species and pycnogenol. Industr. Crops Prod. **2012**, *38*, 115–123.

[38] Grzegorczyk, I.; Matkowski, A.; Wysokinska, H. Antioxidant activity of extracts from in vitro cultures of *Salvia officinalis* L. Food Chem. **2007**, *104*, 536–541.

Downloaded by [York University Libraries] at 10:15 12 August 2014