Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. 74 (10) 1035–1040 (2009)
JSCS–3897

UDC 582.475.2:665.52/.54:
615.282–188(497.11)
Original scientific paper

Chemical composition and antifungal activity of the essential oil of Douglas fir (*Pseudosuga menziesii* Mirb. Franco) from Serbia

VELE TEŠEVIĆ[1]*#, SLOBODAN MILOSAVLJEVIĆ[1#], VLATKA VAJS[2#], IRIS ĐORĐEVIĆ[3], MARINA SOKOVIĆ[4], VERA LAVADINOVIĆ[5] and MIROSLAV NOVAKOVIĆ[2]

[1]*Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade,* [2]*Institute for Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade,* [3]*Faculty for Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, 11000 Belgrade,* [4]*Institute for Biological Research "S. Stanković", University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade and* [5]*Institute of Forestry, Kneza Višeslava 3, 11030 Belgrade, Serbia*

(Received 25 February, revised 8 April 2009)

Abstract: The chemical composition of the essential oil of fresh young needles with twigs of Douglas fir (*Pseudosuga menziesii* Mirb. Franco) obtained by hydrodistillation were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Ten compounds, accounting for 94.26 % of the oil, were identified. The main compounds found were bornyl acetate (34.65 %), camphene (29.82 %), α-pinene (11.65 %) and santene (5.45 %). The antifungal activity of the essential oil was tested against various fungal species. The minimum inhibitory concentration of Douglas fir essential oil ranged from 1.5 to 4 μg mL^{-1}. The fungi most sensitive to the tested oil were *Phomopsis helianthi*, while *Penicillium* species, along with *Microsporum canis*, were the most resistant. Compared to the commercial fungicidal agent bifonazole, the studied essential oil demonstrated higher antifungal activity.

Keywords: antifungal activity; bornyl acetate; Douglas fir; essential oil.

INTRODUCTION

Douglas fir (*Pseudosuga menziesii* Mirb. Franco) is an autochthonous conifer species from North America and Canada, belonging to the family Pinaceae. It is the most introduced species in Europe since 1825 because of its high wood yield, fast growth and wide uses. Douglas fir is a large to very large tree with a narrow, pointed crown of slightly drooping branches, the crown becoming flat-

* Corresponding author. E-mail: vtesevic@chem.bg.ac.rs
# Serbian Chemical Society member.
doi: 10.2298/JSC0910035T

tened with age in large specimens. The needles are petiolate and leave a small, raised scar on the twig.

Douglas fir was often employed medicinally by various native North American Indian tribes, who used it to treat a variety of complaints. An antiseptic resin is obtained from the trunk. It is used as a poultice to treat cuts, burns, wounds and other skin ailments. The resin is used for the treatment of coughs and can be chewed as a treatment for sore throats. An infusion of the green bark has been used in the treatment of excessive menstruation, bleeding bowels and stomach problems. An infusion of the leaves has been used as a wash and a sweat bath for rheumatic and paralyzed joints. An infusion of the young sprouts has been used in the treatment of colds. A decoction of the buds has been used in the treatment of venereal disease. A mouthwash is made by soaking the shoots in cold water. The fresh leaves have a pleasant balsamic odour and are used as a coffee substitute.[1]

Antimicrobial and vermicidal activities of essential oils of Douglas fir against bacteria, fungi and worms are well known.[2–6]

Previous studies of the volatiles from *P. menziesii* include essential oils from Bulgaria,[7,8] Austria,[9] Slovakia[10] and the United States.[11] In the oils from Bulgaria, the main constituents were the monoterpenes *β*-pinene, sabinene, (*Z*)-*β*--ocimene, (*E*)-*β*-ocimene, *α*-terpinolene, *α*-terpineol, citronellyl acetate, *α*-terpinene and limonene.[7,8]

The main constituents of the investigated Austrian samples of *P. menziesii* were *β*-pinene, sabinene (both dominate in samples of needles) and terpinen-4-ol, *α*-pinene, 3-carene, limonene, terpinolene, *α*-terpineol, *α*-terpinene, *γ*-terpinene, and myrcene in twigs.[9] Compounds identified in samples from California included *α*-pinene, camphene, *β*-pinene, 3-carene, myrcene, limonene, 2-hexenal, ethyl caproate, *γ*-terpinene, terpinolene, ethyl caprylate, citronellal, linalool, fenchyl alcohol, bornyl acetate, terpinen-4-ol, *β*-caryophyllene, citronellyl acetate, *α*-terpineol, citronellol, geranyl acetate, farnesyl acetate, *p*-cymene and farnesol.[12]

The composition of the volatiles of Douglas fir depends on seasonal, geographic and ecological conditions. Urban conditions cause notable changes in the *α*-pinene to *β*-pinene, *α*-phellandrene and terpineol ratios *vs*. the relatively clean (arboretum) locality.[10]

The aim of this study was to obtain, for the first time, information on the composition and antifungal activity of the essential oils of fresh needles with twigs of Serbian Douglas fir.

EXPERIMENTAL

Plant material

Needles with twigs of *Pseudosuga menziesii* Mirb. Franco (Pinaceae) were collected from a provenance experiment in Serbia during August 1999 at the mountain Juhor, 745 m. Vou-

cher specimens, accession number PM0899, are deposited in the Herbarium of the Faculty of Biology, University of Belgrade, Herbarium code: BEOU.

The provenance experimental plot in central Serbia on the mountain Juhor (745 m) was established with original material from the native area of the species from Oregon and Washington.

Isolation of the essential oil

Fresh needles with twigs (500 g) were crushed and steam distilled in a Clevenger apparatus for about four hours to obtain a yellow coloured oil (yield: 0.67 %).

Analysis of the essential oil

Gas chromatographic analysis was performed using an HP 5890 gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector. The separation was achieved using a HP-5 (5 % diphenyl- and 95 % dimethylpolysiloxane) fused silica capillary column, 30 m×0.25 mm i.d., 0.25 μm film thickness. The GC oven temperature was programmed from 50 °C (6 min) to 285 °C at a rate of 4.3 °C/min. Hydrogen was used as the carrier gas; flow rate: 1.6 mL/min at 45 °C; injector temperature: 250 °C; detector temperature: 280 °C; injection mode: splitless.

Gas chromatographic–mass spectrometric analysis (EI) was performed using an Agilent 5973 Network chromatograph coupled to an Agilent 5973 MSD spectrometer. The separation was achieved using an Agilent 19091S-433 HP-5MS fused silica capillary column, 30 m×0.25 mm i.d., 0.25 μm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 4.3 °C/min. Helium was used as the carrier gas; the inlet pressure was 25 kPa; the linear velocity was 1ml/min at 210 °C; injector temperature: 250 °C; injection mode: splitless. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; energy, 70 eV; mass scan range, 40–350 amu.

The components were identified based of their retention index and comparison with reference spectra (Wiley and NIST databases). The percentage (relative) of the identified compounds was computed from their GC peak area.

Antifungal activity

The fungi used in this study were *Aspergillus niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *A. versicolor* (ATCC 11730), *A. flavus* (ATCC 9170, *A. terreus* (ATCC 16792), *Alternaria alternata* (ATCC 13963), *Penicillium ochrochloron* (ATCC 9112), *P. funiculosum* (ATCC 10509), *Cladosporium cladosporioides* (ATCC 13276), *Trichoderma viride* (IAM 5061), *Fusarium tricinctum* (CBS 514478) and *Phomopsis helianthi* (ATCC 201540). Among tested species were dermatomycetes (*Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis*), which are obtained directly from patients at the Centre for Preventive Medicine, VMA, Belgrade. The organisms were obtained from the Mycotheca of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research “Dr. Siniša Stanković”, Belgrade. The fungi were maintained on potato dextrose agar (PDA) and malt agar (MA). The cultures were stored at +4 °C and subcultured once a month. In order to investigate the antifungal activity, a modified mycelial growth test with malt agar was used. The minimal inhibitory concentration (*MIC*) of the investigated essential oil necessary for complete inhibition of mycelial growth of the fungal strain was determined. Different concentrations of the tested essential oils were diluted in Petri dishes with malt agar (MA). All experiments were performed in triplicate. Petri dishes with ethanol were used as the control. The compound was added into molten malt agar (MA) and poured into Petri dishes. The tested fungi were inoculated at the centre of the plates.[13] The plates were incubated for

three weeks at room temperature. After this period, the *MIC* was determined. Commercially available bifonazole, Srbolek, Belgrade, was used as the positive control.

RESULTS AND DISCUSSION

The yield of essential oil isolated by hydrodistillation of the needles with twigs of *P. menziesii* was found to be 0.67 % (w/w) based on fresh material.

The constituents identified by GC and GC/MS analysis and the area percenttages are summarized in Table I. The quantitative composition of oil was analyzed by GC (FID) by internal normalization assuming an identical mass response factor for all compounds. In this study, only those components present in the oils in amounts higher than 0.1 % were taken into consideration.

TABLE I. Chemical composition of *Pseudosuga menziesii* essential oil

Compound	*RI*[a]	Content, %
Santene	888	5.45
Tricyclene	926	2.94
α-Pinene	939	11.65
Camphene	953	29.82
β-Pinene	980	2.73
Limonene	1031	4.51
Citronellal	1151	0.94
Bornyl acetate	1285	34.65
Citronellol acetate	1354	0.21
Myrtanol acetate	1381	1.36
Total		94.26

[a]Retention index on HP-5MS and according to *n*-paraffins

Monoterpene hydrocarbons were the major constituents of the oil. In addition to bornyl acetate (34.65 %), which was the major monoterpene, camphene (29.82 %), α-pinene (11.65 %), santene (5.45 %) and limonene (4.52 %) were present in quite high amounts. Most of the components identified in the Douglas fir oil were previously reported as being present in other conifers. Bornyl acetate was also the main constituent of the essential oil of *P. menziesii* from Slovakia[10] and the Rocky Mountains (USA).[11] The essential oil from Bulgaria contained β-pinene (24.4 %), sabinene (22.2 %) and α-terpinolene (18.8 %).[5] The main constituents of the essential oil from Austrian Douglas fir were β-pinene and sabinene.[9]

The essential oil showed significant antifungal potential against the various tested micro-organisms (Table II). The minimum inhibitory concentration (*MIC*) of the oil against *Phomopsis helianthi* was 1.5 μL mL^{-1}. The highest *MIC* (6 μL mL^{-1}) of the oil was against *Penicillium ochrochloron*, *Penicillium funiculosum* and *Microsporum canis*. The commercial fungicide, bifonazole, showed lower antifungal activity than the *P. menziesii* oil, with an *MIC* of 8.0–15.0 μL mL^{-1}. Based on the obtained results, it can be concluded that, compared to the com-

mercial fungicide, the investigated essential oil demonstrated higher potential against various pathogenic fungi.

TABLE II. Minimal inhibition concentration (μL mL^{-1}) of *Pseudosuga menziesii* essential oil and bifonazole

Fungi	Oil	Bifonazole[a]
Alternaria alternata	2.7±0.6	9.6±0.6
Aspergillus niger	3.87±0.3	9.6±0.6
Aspergillus ochraceus	3.87± 0.3	9.3±1.1
Aspergillus versicolor	3.7±0.6	9.6±0.6
Aspergillus flavus	3.7±0.3	9.3±1.1
Aspergillus terreus	3.7±0.6	10±0
Cladosporium cladosporioides	3.0±0	9.6±0.6
Fusarium tricinctum	3.0±0	9.6±0.6
Penicillium ochrochloron	6.0±1	14.3±1.2
Penicillium funiculosum	6.0±1	14.3±1.2
Phomopsis helianthi	1.5±0.5	7.6±0.6
Trichoderma viride	3.7±0.6	14.7±0.6
Trichophyton mentagrophytes	2.7±0.6	10±0
Microsporum canis	6.0±0	14.7±0.6
Epidermophyton floccosum	3.7±0.6	9.6±0.6

[a]Positive control, commercial preparation containing 1.0 % (w/v) of bifonazole in ethanol

Acknowledgments. This research was supported by a grant from the Ministry of Science and Technological Development of the Republic of Serbia (Project 142053).

ИЗВОД

ХЕМИЈСКИ САСТАВ И АНТИФУНГАЛНА АКТИВНОСТ ЕТАРСКОГ УЉА ДАГЛАСОВЕ ЈЕЛЕ (*Pseudosuga Menziesii* Mirb. Franco) ИЗ СРБИЈЕ

ВЕЛЕ ТЕШЕВИЋ[1], СЛОБОДАН МИЛОСАВЉЕВИЋ[1], ВЛАТКА ВАЈС[2], ИРИС ЂОРЂЕВИЋ[3], МАРИНА СОКОВИЋ[4], ВЕРА ЛАВАДИНОВИЋ[5] и МИРОСЛАВ НОВАКОВИЋ[2]

[1]Хемијски факултет, Универзитет у Београду, Студентски трг 16, 11000 Београд, [2]Институт за хемију, технологију и металургију, Универзитет у Београду, Његошева 12, 11000 Београд, [3]Факултет ветеринарске медицине, Универзитет у Београду, Булевар ослобођења 18, 11000 Београд, [4]Институт за биолошка истраживања "С. Станковић", Универзитет у Београду, Булевар деспота Стефана 142, 11000 Београд и [5]Институт за шумарство, Кнеза Вишеслава 3, 11030 Београд

Хемијски састав етарског уља младих иглица са гранчицама дуглазије (*Pseudosuga menziesii* Mirb. Franco) добијеног дестилацијом воденом паром, анализиран је гасном хроматографијом (GC) и комбинацијом гасне хроматографије и масене спектрометрије (GC/MS). Идентификовано је десет једињења укупне заступљености 94,26 %. Као главне компоненте су нађени: борнил-ацетат (34,65 %), камфен (29,82 %), α-пинен (11,65 %) и сантен (5,45 %). Поред тога, етарско уље је тестирано на антифунгалну активност. Етарско уље показује много бољу антифугалну акивност од комерцијалног фунгицидног агенса бифоназола. Минимална инхибиторна концентрација етрског уља дуглазије је у опсегу од 0,6 до 1,4 μL mL^{-1}. Најосетљивији на тестирано уље је био сој *Phomopsis helianthi*, док сојеви *Penicillium* и *Microsporum canis* показују највећу резистентност.

(Примљено 25. фебруара, ревидирано 8. априла 2009)

REFERENCES

1. D. E. Moerman, *Native American Ethnobotany*, Timber Press, Portland, OR, 1998
2. J. Zou, R. G. Cates, *J. Chem. Ecol.* **21** (1995) 387
3. J. Zou, R. G. Cates, *J. Chem. Ecol.* **23** (1997) 2313
4. J. C. Chalchat, R. Ph. Garry, P. Bastide, F. Fabre, R. Malhuret. *Plant Med. Phytother.* **25** (1991) 184
5. L. Jirovetz, G. Buchbauer, A. Stoyanova, S. Metodiev, *Sci. Pharm.* **68** (2000) 323
6. W. H. Johnston, J. J. Karchesy, G. H. Constantine, A. M. Craig, *Phytother. Res.* **15** (2001) 586
7. L. Jirovetz, C. Puschmann, A. Stojanova, S. Metodiev, G. Buchbauer, *Flavour Fragr. J.* **15** (2000) 434
8. A. Stoyanova, S. Metodiev, L. Jirovetz, G. Buchbauer, *Recent Res. Dev. Agric. Food Chem.* **5** (2001) 149
9. G. Buchbauer, L. Jirovetz, M. Wasicky, A. Nikiforov, *J. Agric. Food Chem.* **42** (1994) 2852
10. J. Supuka, F. Berta, *Ekologia* **17** (1998) 102
11. E. von Rudloff, *Can. J. Bot.* 50 (1972) 1025
12. T. Sakai, H. Maarse, R. Kepner, W. G. Jennings, W. M. Longhurst, *J. Agric. Food Chem.* **15** (1967) 1070
13. H. Hanel, W. Raether, *Mycoses* **31** (1988) 154.