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

J. Serb. Chem. Soc. 72 (11) 1045–1051 (2007) UDC **Cotinus coggygria*:665.52/.54+615.281/.282(497.11)
JSCS–3638 *Original scientific paper*

Chemical composition, antibacterial and antifungal activity of the essential oils of *Cotinus coggygria* from Serbia

MIROSLAV NOVAKOVIĆ[1#], IVAN VUČKOVIĆ[1#], PEĐA JANAĆKOVIĆ[2], MARINA SOKOVIĆ[3], ANKA FILIPOVIĆ[4], VELE TEŠEVIĆ[5#] and SLOBODAN MILOSAVLJEVIĆ[5#*]

[1]Institute for Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, [2]Faculty of Biology, Studentski trg 16, 11001 Belgrade, [3]Institute for Biological Research "Siniša Stanković", Bulevar Despota Stefana 142, 11000 Belgrade, [4]Institute of Public Health, Bulevar Despota Stefana 54a, 11000 Belgrade and [5]Faculty of Chemistry, Studentski trg 16, 11001 Belgrade, Serbia

(Received 19 December 2006, revised 6 July 2007)

Abstract: Essential oils from leaves with young branches of *Cotinus coggygria* Scop. from two localities in Serbia (Deliblatska peščara and Zemun), obtained by hydrodistillation, were analysed by GC–MS. Thirty-one component were identified from both oils and among them monoterpenic hydrocarbons were the dominant class (87.4 and 93.1 %). The dominant constituent in both essential oils was limonene (47.0 and 39.2 %). Both oils were also tested for antibacterial and antifungal activities. In comparison to streptomycin, both oils showed slightly higher activity (against most Gram-positive bacteria) in the disc diffusion method and slightly lower activity when the microdilution method was employed. They also exhibited antifungal potential higher than that of the commercial fungicide bifonazole.

Keywords: *Cotinus coggygria*, essential oil composition, antibacterial activity, antifungal potential.

INTRODUCTION

Cotinus is a small genus of the family Anacardiaceae with two species: *Cotinus coggygria* Scop., Eurasian smoketree, and *Cotinus obovatus* Raf., American smoketree.[1] The flora of Serbia comprises two varieties of *C. coggygria*: var. *laevis* and var. *arenaria*. *C. coggygria* is a deciduous, polygamous shrub or little tree up to 7 m tall. It has a wide distribution from southern Europe, the Mediterranean, Moldova, and the Caucasus to central China and the Himalayas. It is frequent and locally common in some parts of Serbia, especially on limestone and sedimentary rocks and in the forests of black hornbeam and black pine.[2] The Serbian local names for this plant are "rujevina" and "ruj", which indicate the dark red colour of the leaves in autumn. In the folk medicine of various countries, the leaves are used as antiseptic, anti-inflammatory, antimicrobial, anti-haemorrhagic,

Serbian Chemical Society member.
* Corresponding author. E-mail: smilo@chem.bg.ac.yu
doi: 10.2998/JSC0711045N

wound-healing and against diarrhoea.[3] The leaves and the hardwood are used for the dyeing of leather, wool and silk into a yellowish colour.[2] Extracts of *C. coggygria* from the USA showed *in vitro* anti-oxidant activity.[4] The leaves and young branches are utilized for the production of essential oil with a terpenic odour for use in perfumery.[5] The antibacterial and antifungal activity of the essential oils of *C. coggygria* have hitherto not been significantly investigated. The essential oil of *C. coggygria* from Hungary inhibited the growth of bacteria (especially Gram-positive strains) and some fungi.[6]

Previous studies of the volatiles from *C. coggygria* included essential oils from Turkey, Bulgaria, Hungary and Greece.[3,5–7] In the oils from Turkey, the main constituents were limonene 48.5 %, (*Z*)-*β*-ocimene 27.9 % and (*E*)-*β*-ocimene 9.7 %.[3] In the oils from Bulgaria, the main components were *α*-pinene 44.0 %, limonene 20.0 %, *β*-pinene 11.4 %.[5] In the oils from Hungary, the main constituents were limonene 30.0–40.0 %, *α*-pinene 24.4–34.3 %, *β*-pinene 7.6–20.2 %, Δ^3-carene 4.6–11.0 % and *α*-terpinolene 3.3–10.6 %.[6] In the oils from Greece, the main components were different in different samples: in the first oil, the main constituents were limonene 67.4 %, *α*-pinene 14.7 % and terpinolene 8.6 %; in the second, myrcene 32.0 %, sabinene 18.0 % and *α*-pinene 15.9 %; in the third oil, main components were sabinene 24.2 %, myrcene 14.0 %, limonene 10.9 % and terpin-4-ol 10.9 %.[7]

In this work, the analyses of two essential oils, both obtained from the leaves with young twigs of wild-growing *C. coggygria* from two localities in Serbia, as well as the antifungal and antibacterial activity of the oils are reported.

EXPERIMENTAL

Plant material. The plant material was collected in June 2005 in Serbia at two localities: Deliblatska peščara (Deliblato Sand) and Zemun.

Isolation of the essential oils. The fresh leaves and twigs were cut up together into small pieces and subjected to hydrodistillation for 2 h using a Clevenger apparatus to obtain the essential oils, having a characteristic terpene-like smell, in yields of 0.23 % (w/w) for the essential oil from Deliblatska peščara and 0.20 % (w/w) for the essential oil from Zemun.

Analysis of the essential oils. The oils were analyzed by GC–FID and GC–MS. The GC analysis was carried out on a gas chromatograph HP 5890 Series II (Hewlett–Packard, USA) fitted with an FID (300 °C), split/splitless injector (250 °C) and an HP-5MS column (30 m×0.25 mm×0.25 μm film thickness). The temperature program was 50–285 °C at a rate of 4.3 °C min^{-1} and the carrier gas was helium (1 ml min^{-1}) measured at 210 °C.

Gas chromatographic–mass spectrometric analysis (GC–MS) was performed using an Agilent 6890 gas chromatograph (Agilent, USA) coupled with an Agilent 5973 Network mass selective detector (MSD) (Agilent, USA), operating in the positive ion electron impact (EI) mode. 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 to 285 °C at a rate of 4.3 °C min^{-1}. Helium was used as the carrier gas, the inlet pressure was 25 kPa, the linear velocity was 1 ml min^{-1} at 210 °C. The injector temperature was 250 °C, and the injection mode splitless. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; *E* energy, 70 eV; mass scan range, 40–350 amu (atomic mass units).

Identification of components. A library search and mass spectral deconvolution and extraction were performed using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification

System) software version 2.4 using retention index (*RI*) calibration data analysis parameters with a "strong" level and 7 % penalty for compounds without a *RI*. The retention indices were experimentally determined using the standard method[6] involving retention times of *n*-alkanes, injected after the essential oil under the same chromatographic conditions. The search was performed using our own library, containing 4951 spectra.

Antibacterial activity. The tests were performed against the following bacteria: *Bacillus cereus* (isolated from food), *Bacillus subtilis* (ATCC 10707), *Escherichia coli* (ATCC 35218), *Micrococcus flavus* (ATCC 10240), *Micrococcus luteus* (ATCC 9341), *Proteus mirabilis* (isolated from food), *Salmonella typhimurium* (ATCC 13311), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus faecalis* (ATCC 25922).

Disc diffusion test. Oils were investigated by disc diffusion using 4 mm filter discs. Bacterial species were cultured overnight at 28 °C in LB (Luria broth) medium. The bacterial suspension containing 10^5 cell/ml was added to the top agar and then dissolved in Petri dishes with solid Muller–Hinton medium. Filter discs with 5 µl of essential oil and another one with 5 µl of streptomycin solution (stock solution in DMSO, concentration of 1 mg ml^{-1}) per disc were placed on the agar plates. After 24 h of incubation at the appropriate temperature, the diameter of the growth inhibition zones was measured.[8] Streptomycin (Sigma P 7794, USA) was used as a positive control.

The minimum inhibitory and minimum bactericidal concentrations, *MIC* and *MBC*, respectively, were determined using 96-well microtitre plates. Series of different concentrations of the tested oils (within the range of 1.25–5.00 µl/ml) were prepared by dissolving in LB medium inoculated with bacterial suspension in saline solution, with a cell concentration of 10^5 cell/ml. The microplates were incubated for 24 h at 28 °C. The lowest concentrations without visible growth (under a binocular microscope) were defined as the concentrations that completely inhibited bacterial growth (*MIC*s). The minimum bactericidal concentrations (*MBC*s) were determined by serial subcultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation for 72 h. The lowest concentration that killed the bacteria was defined as the *MBC*, indicating 99.5 % killing of the original inoculums. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with the blank and positive control. Commercial antibiotic, streptomycin (stock solution in DMSO, concentration of 1 mg ml^{-1}) was used as the positive control.[9]

Antifungal activity. In order to investigate the antifungal activity of the essential oils of *C. coggygria*, a modified microdilution technique was used.[10] The fungal spores were washed from the surface of agar plates with sterile 0.85 % saline containing 0.1 % Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µl. The tests were performed against the following fungal species: *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigatus* (isolated from animals), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 10509), *Trichoderma viride* (IAM 5061), *Candida albicans* (clinical isolate) and *Trichophyton mentagrophytes* (clinical isolate). The commercial fungicide bifonazole (Srbolek, Serbia) concentration of 1 % (w/v) was used as the positive control.

The minimum inhibitory concentrations (*MIC*s) determination was performed by the dilution technique using 96-well microtitre plates. The tested essential oils were dissolved in broth MA (Malt agar) medium; the final concentration range was 1.25–20.00 µl/ml. The microplates were incubated for 72 h at 28 °C. The lowest concentration without visible growth (under a binocular microscope) was defined as the concentration which completely inhibited fungal growth (*MIC*), while those that killed the fungi were the minimal fungicidal concentrations (*MFC*). The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories, USA) and compared with the blank and the positive control.

RESULTS AND DISCUSSION

The yields of the oils of both samples of *C. coggygria*, calculated per weight of fresh plant material (leaves and branches), were 0.23 % (w/w) for oil from Deliblatska peščara and 0.20 % (w/w) for oil from Zemun. 21 and 22 Components were identified in each of the oils from Deliblatska Peščara and Zemun, respectively. Both oils showed very similar chemical composition with monoterpenic hydrocarbons dominating (87.4 and 93.1 %, respectively) The major components, *i.e.*, limonene (47.0 and 39.2 %), (*Z*)-*β*-ocimene (16.4 and 26.3 %), *α*-pinene (8.2 and 8.4 %), (*E*)-*β*-ocimene (4.6 and 9.0 %) and terpinolene (6.8 and 5.3 %) were the same in both oils (Table I). Limonene was also the main constituent of the essential oils of *C. coggygria* from Turkey,[3] Hungary[6] and Greece (Mt Pilio).[7] The essential oil from Bulgaria contained *α*-pinene (44 %) as the main component and a high percentage of limonene (20 %).[5] High percentage of (*Z*)- and (*E*)-*β*-ocimenes (21 and 35.3 %), present in a similar ratio (*Z*:*E ca*. 3:1) to that observed in the oil of Turkish origin,[3] is another characteristic of these oils.

TABLE I. Composition of the studied *C. coggygria* essential oils

Components	*RRI*[a]	Deliblatska peščara[b]	Zemun[c]
(*Z*)-3-Hexenal	800	0.4	–
(*E*)-2-Hexenal	854	7.6	1.2
Tricyclene	926	t	–
α-Pinene	939	8.2	8.8
α-Fenchene	951	t	–
Camphene	953	–	1.0
β-Pinene	980	2.1	2.1
β-Myrcene	991	1.5	1.4
α-Phellandrene	1005	0.4	–
(*Z*)-Hex-3-en-1-ol acetate	1007	t	–
Δ^3-Carene	1011	–	t
Limonene	1031	47.0	39.2
(*Z*)-*β*-Ocimene	1040	16.4	26.3
(*E*)-*β*-Ocimene	1050	4.6	9.0
γ-Terpinene	1062	t	t
Terpinolene	1088	6.8	5.3
p-Cymenene	1089	t	–
Linalool	1098	t	–
Alloocimene	1129	0.4	1.4
α-Terpineol	1189	t	t
Bornyl acetate	1285	–	t
β-Caryophyllene	1418	1.1	2.5
α-Humulene	1454	–	t

TABLE I. Continued

Components	*RRI*	Deliblatska peščara	Zemun
γ-Muurolene	1477	0.8	t
Germacrene D	1480	–	0.5
Bicyclogermacrene	1494	–	–
γ-Cadinene	1513	–	t
δ-Cadinene	1524	0.4	0.7
Caryophyllene oxide	1581	–	t
α-Cadinol	1653	–	t
Total		97.3	99.4

[a]*RRI*, relative retention indices calculated against C_x–C_y *n*-alkanes under the same GC conditions; [b]oil yield 0.23 % (w/w); [c]oil yield 0.20 % (w/w); t – concentration less than 0.4 %

The antibacterial activity obtained by the disc diffusion method is presented in Table II. The essential oil from Deliblatska peščara showed inhibition zones from 6–23 mm. The highest zones were obtained against the *Staphylococcus* and *Micrococcus* species, while the lowest activity was against *Proteus mirabilis*. Inhibition zones of 6–28 mm were obtained for the oil from Zemun, with slightly higher activity against *Staphylococcus* species than of the oil from Deliblatska peščara. Both oils exhibited the same actitivity against *Micrococcus* species, while *Staphylococcus* and *Micrococcus* species showed higher sensitivity than other bacteria species. According to these tests, both oils showed higher antibacterial activity than streptomycin used as the positive control, except in the case of *P. mirabilis*.

TABLE II. Diameters of the inhibition zones (mm) in the disc diffusion method of determining the antibacterial activity of the studied *C. coggygria* essential oils (5 µl/disc)

Bacteria	Deliblatska peščara	Zemun	Streptomycin[a]
Bacillus cereus	15	18	10
Bacillus subtilis	15	17	10
Escherichia coli	9	10	10
Micrococcus flavus	22	23	22
Micrococcus luteus	20	20	20
Proteus mirabilis	6	6	8
Salmonella typhimurium	9	11	12
Staphylococcus aureus	23	28	15
Staphylococcus epidermidis	19	25	12
Staphylococcus faecalis	23	27	14

[a]Positive control, streptomycin, concentration 1 mg ml^{-1} (in DMSO)

The results of antibacterial activity (*MIC* and *MBC*) are presented in Table III. The oil from Deliblatska peščara showed lower antibacterial activity in this test with bacteriostatic activity in the concentration range 2.5–5.0 µl/ml, while bactericidal concentrations were in the range of 2.5–10.0 µl/ml. The essential oil from

Zemun showed activity with *MIC* and *MBC* values ranging from 1.25–5.0 µl/ml. *P. mirabilis* showed a higher resistance than other bacteria in this test, while *Salmonella typhymurium*, the *Bacillus* species and *Escherichia coli* showed higher sensitivity to the tested oils. The commercial antibiotic, streptomycin, showed slightly better antibacterial activity than the two essential oils.

TABLE III. Antimicrobial activity expressed as minimum inhibitory (*MIC*) and minimum bactericidal concentrations (*MBC*), in µl/ml, of the studied *C. coggygria* essential oils, determined by the microdilution method

Bacteria	Deliblatska peščara		Zemun		Streptomycin[a]	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*[b]	*MBC*[b]
Bacillus cereus	2.5	2.5	1.25	1.25	0.25	0.5
Bacillus subtilis	2.5	2.5	1.25	1.25	0.25	0.5
Escherichia coli	5.0	5.0	1.25	1.25	0.5	1.0
Micrococcus flavus	2.5	2.5	2.5	2.5	1.0	2.0
Micrococcus luteus	2.5	2.5	2.5	2.5	1.0	2.0
Proteus mirabilis	5.0	10.0	2.5	5.0	1.0	1.0
Salmonella typhimurium	2.5	2.5	1.25	1.25	0.25	0.5
Staphylococcus aureus	2.5	2.5	1.25	2.5	1.0	2.0
Staphylococcus epidermidis	2.5	2.5	2.5	2.5	1.0	2.0
Staphylococcus faecalis	2.5	2.5	1.25	2.5	1.0	2.0

[a]Positive control, streptomycin concentration 1 mg ml^{-1} (in DMSO); [b]in µg ml^{-1}

The antifungal activity of the essential oils is presented in Table IV. It can be noticed that the oil from Deliblatska peščara showed antifungal activity with *MIC* values of 5.0–40.0 µl/ml and *MFC* values of 10.0–40.0 µl/ml. The antifungal activity of the oil from Zemun was even better with *MIC* values between 1.25–10.0 µl/ml and *MFC* values of 2.5–20.0 µl/ml. *Trichoderma viride* showed higher resistance to both oils, while *Candida albicans* and *Trichophyton mentagrophytes* were more sensitive than the other fungi. The commercial fungicide, bifonazole, used as the positive control showed activity with higher *MIC* and *MFC* values than the essential oils.

TABLE IV. Minimum inhibitory (*MIC*) and minimum fungicidal concentrations (*MFC*), in µl/ml, of the studied *C. coggygria* essential oils, determined by the microdilution method

Micromycetes	Deliblatska peščara		Zemun		Bifonazole[a]	
	MIC	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*
Aspergillus niger	10.0	10.0	2.5	5.0	20.0	20.0
Aspergillus ochraceus	10.0	10.0	2.5	5.0	20.0	20.0
Aspergillus versicolor	5.0	10.0	2.5	5.0	20.0	20.0
Aspergillus flavus	10.0	20.0	5.0	10.0	20.0	20.0
Aspergillus fumigatus	10.0	20.0	5.0	10.0	40.0	40.0
Penicillium ochrochloron	20.0	20.0	5.0	10.0	40.0	80.0
Penicillium funiculosum	20.0	20.0	5.0	10.0	40.0	80.0

TABLE IV. Continued

Micromycetes	Deliblatska peščara		Zemun		Bifonazole	
	MIC	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*
Trichoderma viride	40.0	40.0	10.0	20.0	80.0	80.0
Candida albicans	5.0	10.0	1.25	2.5	20.0	20.0
Trichophyton mentagrophytes	5.0	10.0	1.25	2.5	20.0	20.0

[a]Positive control, commercial preparation containing 1 % (w/v) of bifonazole in ethanol.

Acknowledgement: The authors are grateful to the Ministry of Science of Serbia (Project 142053) for financial support.

И З В О Д

ХЕМИЈСКИ САСТАВ, АНТИБАКТЕРИЈСКА И АНТИФУНГАЛНА АКТИВНОСТ ЕТАРСКИХ УЉА БИЉНЕ ВРСТЕ *Cotinus coggygria* ИЗ СРБИЈЕ

МИРОСЛАВ НОВАКОВИЋ[1], ИВАН ВУЧКОВИЋ[1], ПЕЂА ЈАНАЋКОВИЋ[2], МАРИНА СОКОВИЋ[3], АНКА ФИЛИПОВИЋ[4], ВЕЛЕ ТЕШЕВИЋ[5] и СЛОБОДАН МИЛОСАВЉЕВИЋ[5]

[1]Институт за хемију, технологију и металургију, Његошева 12, 11000 Београд, [2]Биолошки факултет, Студентски трг 16, 11001 Београд, [3]Институт за биолошка истраживања "Синиша Станковић", Булевар Деспота Стефана 142, 11000 Београд, [4]Градски Завод за заштиту здравља, Булевар Деспота Стефана 54а, 11000 Београд и [5]Хемијски факултет, Студентски трг 16, 11001 Београд

Применом GC–MS методе идентификована је укупно 31 компонента у оба етарска уља изолована дестилацијом воденом паром из лишћа и младих гранчица биљне врсте *Cotinus coggygria* Scop. са два локалитета у Србији (Делиблатска пешчара и Земун). Такође је испитана антибактеријска и антифунгална активност оба узорка. У оба уља најзаступљенији су били монотерпенски угљоводоници (87,4 и 93,1 %), међу којима је доминантна компонента лимонен (47,0 и 39,2 %). Антибактеријска активност је одређена дифузионом и микродилуционом методом, а антифунгална активност модификованом микродилуционом методом. Према дуфузионој методи, оба уља су показала нешто боље антибактеријско дејство (првенствено на грам-позитивне бактерије) него стрептомицин, док је на основу микродилуционе методе њихова активност била нешто слабија. У поређењу са бифоназолом, оба тестирана уља су показала нешто боље антифунгално дејство.

(Примљено 19. децембра 2006, ревидирано 6. јула 2007)

REFERENCES

1. http://en.Wikipedia.org./wiki/Cotinus (May 10, 2006)
2. M. Gajić, in *Flora of Serbia V*, M. Josifović, Ed., SANU, Belgrade 1973, p. 57
3. B. Demirci, F. Demirci, K. H. C. Baser, *Flavour Fragr. J.* **18** (2003) 43
4. H. E. Westenburg, K. J. Lee, S. K. Lee, *J. Nat. Prod.* **63** (2000) 1696
5. E. T. Tsankova, A. S. Dyulgerov, B. K. Milenkov, *J. Essent. Oil Res.* **5** (1993) 205
6. I. Hethelyi, J. Domokos, E. Lemberkovics, G. Verzar–Petri, *Herba Hung.* **25** (1986) 135
7. O. Tzakou, I. Bazos, A. Yannitsaros, *Flavour Fragr. J.* **20** (2005) 531.
8. H. Van Den Dool, P. D. Kratz, *J. Chromatogr.* **11** (1963) 4637
9. R. Verpoorte, T. A. Van Beek, P. H. A. M. Thomassen, J. Andeweil, A. Baerhim Svendsen, *Ethnopharmacol.* **8** (1983) 2878
10. H. Hanel, W. Raether, *Mycoses* **31** (1988) 148
11. K. D. Daouk, M. S. Dagher, J. E. Sattout, *J. Food Prot.* **58** (1995) 1147.