

Original Article

## Antimicrobial Activities of Essential Oil and Methanol Extract of *Teucrium montanum*

Nenad Vukovic, Tanja Milosevic, Slobodan Sukdolak and Slavica Solujic

Department of Chemistry, Faculty of Science, University of Kragujevac, PO Box 12, Serbia

This study was designed to examine the chemical composition of essential oil and the *in vitro* antimicrobial activities of essential oil and methanol extract of *Teucrium montanum*. The inhibitory effects of essential oil and methanol extracts of *T. montanum* were tested against 13 bacterial and three fungal species by using disc-diffusion method. GC/MS analyses revealed that essential oil contains mainly  $\delta$ -cadinene (17.19%),  $\beta$ -selinene (8.16%)  $\alpha$ -calacorene (4.97%), 1,6-dimethyl-4-(1-methylethyl)-naphthalene (4.91%), caryophyllene (4.35%), copaene (4.23%), torreyol (3.91%), 4-terpineol (3.90%), cadina-1,4-diene (3.39%),  $\beta$ -sesquiphellandrene (3.34%),  $\tau$ -cadinol (3.12%) and  $\gamma$ -curcumene (3.18%). The essential oil has antibacterial as well as antifungal effect.

**Keywords:** antimicrobial activity – essential oil – *Teucrium montanum*

### Introduction

The use of phytochemicals as natural antimicrobial agents commonly called “biocides” is gaining popularity (1). There is growing interest in correlating phytochemical constituents of plant with its pharmacological activity (2). The antimicrobial properties of essential oils have been recognized for many years, and their preparations have found applications as naturally occurring antimicrobial agents in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation, etc. The essential oil preparations that possess antimicrobial activities have been the subject of many investigations resulting in the screening of a wide variety of plant species, and have revealed structurally unique biologically active compounds. However, less attention was given to the activities of their main components in the oils tested. The main advantage of natural agents is that they do not enhance the “antibiotic resistance”, a phenomenon commonly

encountered with the long-term use of synthetic antibiotics. There are reports of the active principles of essential oils from various plants with antibacterial or antifungal activity. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds (thymol, carvacrol, eugenol), which also in pure form demonstrate high antibacterial activity (3). The essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Gram-negative bacteria were shown to be generally more resistant than Gram-positive ones to the antagonistic effects of essential oils because of the lipopolysaccharide present in the outer membrane, but this was not always true.

*Teucrium montanum* is a grass crop that has long been consumed both as a herbal medicine and as a nourishing food. It is widely used as diuretic, stomachic, analgesic and antispasmodic agent, and also possesses antibacterial, antifungal, anti-inflammatory and antioxidative activity [Jancic *et al.* (4), Tumbas *et al.* (5)].

The objective of the present study was to identify the constituents of the essential oil of *T. montanum* and to carry out an evaluation of their antimicrobial activity with their chemical composition.

For reprints and all correspondence: Nenad Vukovic, Department of Chemistry, Faculty of Science, University of Kragujevac, PO Box 12, Serbia. Tel: +38134336223; Fax: +38134336085; E-mail: nvukovic@kg.ac.yu

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## Material and Methods

### Plant Material and Isolation of the Essential Oil

*Teucrium montanum* was collected from mountain Jadovnik in August 2006. The species was identified, and the voucher specimen was deposited (16177, BEOU, Snežana Vukojičić) at Department of Botany, Faculty of Biology, University of Belgrade. The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus for 3 h, from aerial parts of *T. montanum*. The oil obtained was dried over anhydrous sodium sulfate overnight and kept in sterile sample tubes in refrigerator. The oil yields were calculated on a dry weight basis as 0.47%.

### Gas Chromatography-Mass Spectrometry (GC-MS)

Analyses were carried out in a Agilent 6890N (G 1530N) gas chromatograph fitted with a HP-5MS fused silica column (5% phenyl methyl polysiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with an Agilent mass selective detector 5975B (Agilent Technologies, USA) (G 3171A) operated by HP Enhanced ChemStation software, G1701DA MSD ChemStation Rev. D.00.00.38. Oven temperature program: 60–240°C, at 3°C min<sup>-1</sup> (62 min analysis time); injector temperature: 250°C; carrier gas: helium, adjusted to a column velocity of flow 1.1 ml min<sup>-1</sup>; split ratio was 25:1, whereas split flow was 30.7 ml min<sup>-1</sup>, interface temperature: 280°C; standard electronic impact (EI) MS source temperature: 230°C; MS quadrupole temperature: 150°C; mass scan range: 50–500 amu at 70 eV; scan velocity: 3.12 scans s<sup>-1</sup>; resulting EM voltage: 1200 V. One microliter of sample (dissolved in hexane 100% v/v) was injected into the system.

### Identification of Essential Oil Constituents

The identification of the components was based on comparison of their mass spectra with Wiley7Nist database through G1701DA mass spectrum ChemStation or with mass spectra reported in literature. Also, the identification can be assisted by comparison of their retention times and retention indices with authentic samples. Quantitative analysis was performed by means direct peak area integration technique based on the TIC.

### Microbial Strains Used

Test microorganism which were used in this experiment are: *Agrobacterium tumefaciens* (PMFKg-B11), *Azotobacter chlorococcum* (PMFKg-B14), *Bacillus mucoides* (IPH), *Bacillus subtilis* (IPH), *Enterobacter cloacae* (PMFKg-B22), *Erwinia carotovora* (PMFKg-B31),

**Table 1.** Antimicrobial susceptibility pattern of essential (10 μl/disc) oil and methanol extract (300 μg/disc) of *T. montanum*

Microorganism	Inhibition zone diameter (mm) <sup>a,b</sup>		Antimicrobial agent
	Essential oil	Methanol extract	
<b>Gram (+)</b>			
<i>Bacillus mycoides</i>	25 ± 0.5	19 ± 0.5	Amracin (300 μg/ml)
<i>Bacillus subtilis</i>	26 ± 0.5	14 ± 0.3	29 ± 0.5
<i>Staphylococcus aureus</i>	10 ± 0.3	8 ± 0.3	30 ± 0.5
<b>Gram (-)</b>			
<i>Agrobacterium tumefaciens</i>	16 ± 0.4	13 ± 0.5	32 ± 0.5
<i>Azotobacter chlorococcum</i>	24 ± 0.5	28 ± 0.5	34 ± 0.5
<i>Enterobacter cloacae</i>	24 ± 0.5	16 ± 0.3	36 ± 0.5
<i>Erwinia carotovora</i>	18 ± 0.3	19 ± 0.5	31 ± 0.5
<i>Klebsiella pneumoniae</i>	29 ± 0.5	22 ± 0.5	35 ± 0.7
<i>Proteus sp.</i>	–	–	11 ± 0.5
<i>Pseudomonas aeruginosa</i>	–	–	17 ± 0.3
<i>Pseudomonas glycinea</i>	20 ± 0.5	16 ± 0.5	35 ± 0.7
<i>Pseudomonas fluorescens</i>	22 ± 0.5	12 ± 0.3	42 ± 0.7
<i>Pseudomonas phaseolicola</i>	23 ± 0.5	19 ± 0.5	35 ± 0.5
<b>Fungi</b>			Nystatin (300 μg/ml)
<i>Aspergillus niger</i>	9 ± 0.5	–	20 ± 0.5
<i>Fusarium oxysporum</i>	17 ± 0.5	11 ± 0.5	28 ± 0.5
<i>Penicillium canescens</i>	10 ± 0.5	–	22 ± 0.5

<sup>a</sup>Mean value ± SD, n = 3 (the zone of inhibition (in millimeter) including disc of 6 mm in diameter). <sup>b</sup>Solvent controls (methanol) was negative.

*Klebsiella pneumoniae* (PMFKg-B26), *Proteus sp.* (PMFKg-B34), *Pseudomonas aeruginosa* (PMFKg-B37), *Pseudomonas glycinea* (PMFKg-B40), *Pseudomonas fluorescens* (PMFKg-B28), *Pseudomonas phaseolicola* (PMFKg-B29), *Staphylococcus aureus* (PMFKg-B30), *Fusarium oxysporum* (Schlecht), *Aspergillus niger* (Van Teghem) and *Penicillium canescens* (Soop).

All tested bacteria cultures were obtained from Institute for Health Protection (IPH) and Faculty of Agriculture, University of Beograd, Serbia. Laboratory for Microbiology, Department of Biology, Faculty of Science, University of Kragujevac, Serbia confirmed identification of all tested microorganism (PMF-Kg)

### Antimicrobial Analysis

The antimicrobial activity of essential oil and methanolic extract of the plant *T. montanum* was investigated by disc-diffusion method on Mueller-Hinton broth. It was performed using a 24 h old bacterial culture at 37°C reseeded on Nutrient Broth. The fungi were reseeded on potato-glucose agar, on which they developed for 72 h at the room temperature of 20°C under alternating

**Table 2.** Chemical composition of *Teucrium montanum* essential oil

Peak No	t (min)	MS + t <sub>ret</sub> identification	%
1	5.953	$\alpha$ -Pinene	tr
2	6.755	1-ethyl-3-methyl-benzene	tr
3	7.419	Sabinene	tr
4	8.597	$\alpha$ -Terpinene	tr
5	8.889	p-Cimene	0.71
6	10.182	$\gamma$ -Terpinene	0.41
7	12.717	$\beta$ -Phellandrene	tr
8	13.295	(2-Methylprop-1-enyl)-cyclohexa-1,3-diene	tr
9	14.245	5-(1-methylethyl)-bicyclo[3,1,0]hexan-2-one	1.10
10	14.635	unknown	1.73
11	15.074	4-Terpineol	3.90
12	15.910	Myrtenal	0.98
13	16.465	cis-Verbenone	1.09
14	17.964	Carvone	tr
15	19.286	Phellandral	tr
16	19.560	1(7),3,8-o-Menthatriene	tr
17	20.001	p-Cymen-7-ol	tr
18	20.493	Carvacrol	tr
19	22.507	$\alpha$ -Copaene	1.14
20	23.600	$\alpha$ -Cubebene	0.78
21	23.989	$\beta$ -Damascenone	0.43
22	24.247	Zingiberene	1.34
23	25.368	Caryophyllene	4.35
24	26.078	$\alpha$ -Bergamotene	1.11
25	26.392	$\beta$ -Sesquiphellandrene	3.34
26	26.638	unknown	0.69
27	26.753	$\alpha$ -Caryophyllene	1.91
28	26.987	$\beta$ -Farnesene	1.76
29	27.050	Aromadendrene	1.32
30	27.601	unknown	1.54
31	27.874	Copaene	4.23
32	28.004	$\alpha$ -Curcumene	1.74
33	28.075	$\beta$ -Selinene	8.16
34	28.303	(+)-Epi-bicyclosesquiphellandrene	1.64
35	28.418	Isoledene	1.62
36	28.664	$\alpha$ -Muuroolene	1.73
37	28.813	cis- $\alpha$ -Bisabolene	0.53
38	29.007	$\beta$ -Bisabolene	0.71
39	29.167	$\alpha$ -Cedrene	2.90
40	29.242	unknown	1.23
41	29.597	$\delta$ -Cadinene	17.19
42	29.917	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	1.29
43	30.318	$\alpha$ -Calacorene	4.97

(continued)

**Table 2.** Continued

Peak No	t (min)	MS + t <sub>ret</sub> identification	%
44	30.393	Unknown	1.87
45	33.562	Cadina-1,4-diene	3.39
46	34.083	Torreyol	3.91
47	34.243	$\gamma$ -Muuroolene	tr
48	34.523	$\tau$ -Cadinol	3.12
49	35.187	$\gamma$ -curcumene	3.18
50	35.256	1,6-dimethyl-4-(1-methylethyl)-naphthalene	4.91

day-night light conditions. The cultures were adjusted to  $5.6 \times 10^6$  CFU/ml with sterile water. One milliliter of the suspension was added over the plates containing Mueller-Hinton broth to get a uniform microbial growth on both control and test plates. The extract of *T. montanum* were dissolved in methanol (30 mg/ml) and sterilized. Under aseptic conditions, empty sterilized discs (Whatman no. 5, 6 mm diameter) were impregnated with 10  $\mu$ l of the essential oil, methanol extract (300  $\mu$ g/ml), and placed on the agar surface. The plates were left for 30 min at room temperature to allow the diffusion of the oil and extract, and then they were incubated at 37°C. After the incubation period (24 h), the zone inhibition were measured and presented in millimeter. Negative controls were prepared using the same solvents employed to dissolve plant extract. Amracin and Nystatin were used as standard antibiotics for comparison.

## Results

The essential oil was extracted by the hydrodistillation of the dried aerial parts of *T. montanum* and the constituents were analyzed by GC-MS. The oil yields were calculated on a dry weight basis as 0.47%. The essential oil of *T. montanum* was analyzed to determine their constituents (Table 2). As are result of GC/MS analyses, 45 compounds were identified, representing 97.95% of the total. GC/MS analyses of the oil have revealed the occurrence of  $\delta$ -cadinene (17.19%) and  $\beta$ -selinene (8.16%) as the major constituents of essential oil. The compounds  $\alpha$ -calacorene (4.97%), torreyol (3.91%), 1,6-dimethyl-4-(1-methylethyl)-naphthalene (4.91%), copaene (4.23%), 4-terpineol (3.90%), cadina-1,4-diene (3.39%),  $\tau$ -cadinol (3.12%) caryophyllene (3.34%),  $\beta$ -sesquiphellandrene (3.98%) and  $\alpha$ -cedrene (2.90%) were also characterized as a main component in the oil of *T. montanum*. Mono and sesqeterpenes hydrocarbons were the characteristic constituents of the oil of *T. montanum*.  $\alpha$ -Pinene, 1-ethyl-3-methyl-benzene, sabinene, (2-methylprop-1-enyl)-cyclohexa-1,3-diene,  $\beta$ -phellandrene, carvone, phellandral, 1(7),3,8-o-menthatriene, p-cymen-7-ol, carvacrol and  $\gamma$ -muuroolene

were found to be the minor components of *T. montanum* oil in the present study (in trace).

According to the results given in Table 1, the essential oil of *T. montanum* had great antimicrobial activity against all investigated microorganism. The diameters of growth inhibition zone ranged from 16 to 29 mm (including the diameter of the disc—6 mm) with the highest inhibition zone values observed against *K. pneumoniae* (29 mm). The greatest level of resistance showed *A. tumefaciens* and *E. carotovora* (the zone inhibition 16 mm). The essential oil showed greater or similar activity on Gram-positive and Gram-negative bacteria and fungi *F. oxysporum*.

In general, the essential oil showed better activity than the methanol extract. The methanolic extract showed strong antibacterial activity against the bacteria *A. chlorococcum*, inhibition zone is 28 mm. Although both essential oil and methanol extract had similar sizes of the zone of inhibition

for *E. carotovora* (18 and 19 mm, respectively). On all other microorganism the methanol extract showed less activity than essential oil.

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