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The chemical composition of the essential oil and the antibacterial activities of the essential oil and methanol extract of *Teucrium montanum*

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Abstract: This study was designed to examine the chemical composition of the essential oil and the *in vitro* antibacterial activities of the essential oil and methanol extract of *Teucrium montanum*. The inhibitory effects of the essential oil and the methanol extracts of *Teucrium montanum* were tested against thirteen bacterial species using the disc-diffusion method. GC/MS analyses revealed that the essential oil contained mainly sesquiterpenes, such as δ-cadinene (17.19 %), β-selinene (8.16 %) and α-calacorene (4.97 %). The highest activities were obtained with the essential oil of *Teucrium montanum* against *K. pneumoniae*, *B. subtilis*, *B. mycoides*, *E. cloaceae* and *A. chlorococcum*. In addition, comparison of the antibacterial activities of the essential oil and the methanol extract showed that the essential oil exhibited the stronger antibacterial activities.

Keywords: essential oil; *Teucrium montanum*; antibacterial activity.

INTRODUCTION

The use of phytochemicals as natural antimicrobial agents, commonly called "biocides", is gaining in popularity.[1] There is growing interest in correlating the phytochemical constituents of a plant with its pharmacological activity.[2,3] The antimicrobial properties of essential oils have been recognized for many years[4,5] and their preparations have found applications as naturally occurring antimicrobial agents in the fields of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation, *etc*. The preparations of essential oils possessing antimicrobial activities have been the subject of many investigations, which, in the screening of a wide variety of plant species, revealed structurally unique biologically active compounds. However, less attention was

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given to the activities of the main components in the tested oils. The main advantage of natural agents is that they do not enhance "antibiotic resistance", a phenomenon commonly encountered with long-term use of synthetic antibiotics. There are reports of active principles of essential oils from various plants with antibacterial or antifungal activity.[6–12] 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 exhibit high antibacterial activity.[13–15] Essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative[16,17] and Gram-positive bacteria.[18] 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[19] but this was not always the case.[15]

Teucrium montanum is a grass crop which has long been consumed both as an herbal medicine and as a nourishing food. It is widely used as a diuretic, stomachic, analgesic and antispasmodic agent, and also possesses antibacterial, antifungal, anti-inflammatory and anti-oxidative activity.[20–22]

Therefore, the objective of the present study was (a) to examine the chemical composition of the essential oil of *T. montanum* by GC–MS and (b) to evaluate the antibacterial activity of the essential oil and methanolic extract of *T. montanum*.

EXPERIMENTAL

Plant material and isolation of the essential oil

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

Gas chromatography–mass spectrometry (GC–MS)

The analyses were carried out in an Agilent 6890N (G 1530N) (Serial No. CN10702033) gas chromatograph fitted with an 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) (Serial No. US65125280) operated by HP Enhanced ChemStation software, G1701DA MSD ChemStation Rev. D.00.00.38. The operating conditions were as follows: oven temperature: 60–240 °C, at 3 °C min^{-1} (62 min analysis time); injector temperature: 250 °C; carrier gas: helium, adjusted to a column velocity of flow 1.1 ml min^{-1}; split ratio 25:1, whereas the split flow was 30.7 ml min^{-1}; 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^{-1}; resulting EM voltage: 1200 V. One microliter of sample (dissolved in hexane 100 % v/v) was injected into the system.

Identification of the constituents of the essential oil

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 the literature. Also, the identification was assisted by comparison of their retention times with authentic samples. Quantitative analysis was performed by means of the direct peak area integration technique based on the TIC.

Microbial strains used

The test microorganism employed in this study were: *Agrobacterium tumefaciens* (PMFKg-B11), *Azotobacter chlorococcum* (PMFKg-B14), *Bacillus mucoides* (IPH), *Bacillus subtilis* (IPH), *Enterobacter cloaceae* (PMFKg-B22), *Erwinia carotovora* (PMFKg-B31), *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).

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

Antimicrobial analysis

The antibacterial activity of the essential oil and methanolic extract of the plant *Teucrium montanum* was investigated by the disc-diffusion method on Mueller–Hinton broth (NCCLS, 1990).[23] It was performed using a 24 h old bacterial culture at 37 °C reseeded on Nutrient broth. The cultures were adjusted to 5.6×10^{6} CFU ml^{-1} with sterile water. One milliliter of the suspension was added over the plates containing the Mueller–Hinton broth to obtain a uniform microbial growth on both the control and test plates. The extracts of *Teucrium montanum* were dissolved in methanol (30 mg ml^{-1}) and sterilized. Under aseptic conditions, empty sterilized discs (Whatman No. 5, 6 mm diameter) were impregnated with 10 µl of the essential oil, methanol extract (300 µg ml^{-1}) 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 before incubation at 37 °C. After the incubation period (24 h), the zone of inhibition was measured and presented in mm. Negative controls were prepared using the same solvents employed to dissolve the plant extract. Amracin was used as the standard antibiotic for comparison.

RESULTS AND DISCUSSION

The essential oil was extracted by hydrodistillation of the dried aerial parts of *Teucrium montanum* and the constituents were analyzed by GC–MS. The yield of oil was 0.47 %, calculated on a dry weight basis. The essential oil of *Teucrium montanum* was analyzed to determine its constituents (Table I). From the GC–MS analysis, 44 compounds were identified in the oil, representing 97.95 % of the total. GC–MS analysis of the oil showed the presence of δ-cadinene (17.19 %) and β-selinene (8.16 %), as the major constituents of the essential oil. The compounds α-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 %), τ-cadinol (3.12 %), caryophyllene (3.34 %), β-sesquiphellandrene (3.98 %) and α-cedrene (2.90 %) were also identified as significant components in the oil of *T. montanum*. Sesquiterpene hydrocarbons were characteristic constituents of the oil of *T. montanum*. α-Pinene, 1-ethyl-3-methylbenzene, sabinene, 1-(2-methylprop-1-enyl)cyclohexa-1,3-diene, β-phellandrene, carvone, phellandral, 1(7),3,8-*o*-menthatriene, *p*-cymen-7-ol, carvacrol and γ-muurolene were found to be minor components of *T. montanum* oil in this study.

TABLE I. Chemical composition of *Teucrium montanum* essential oil

t / min	MS + t_{ret} identification	Content, %
5.953	α-Pinene	tr
6.755	1-Ethyl-3-methylbenzene	tr
7.419	Sabinene	tr
8.597	α-Terpinene	tr
8.889	*p*-Cimene	0.71
10.182	γ-Terpinene	0.41
12.717	β-Phellandrene	tr
13.295	1-(2-Methylprop-1-enyl)cyclohexa-1,3-diene	tr
14.245	5-(1-Methylethyl)-bicyclo[3.1.0]hexan-2-one	1.1
14.635	Unknown	1.73
15.074	4-Terpineol	3.90
15.910	Myrtenal	0.98
16.465	*cis*-Verbenone	1.09
17.964	Carvone	tr
19.286	Phellandral	tr
19.560	1(7),3,8-*o*-Menthatriene	tr
20.001	*p*-Cymen-7-ol	tr
20.493	Carvacrol	tr
22.507	Unknown	1.14
23.600	α-Cubebene	0.78
23.989	β-Damascenone	0.43
24.247	Zingiberene	1.34
25.368	Caryophyllene	4.35
26.078	α-Bergamotene	1.11
26.392	β-Sesquiphellandrene	3.34
26.638	Unknown	0.69
26.753	α-Caryophyllene	1.91
26.987	β-Farnesene	1.76
27.050	Aromadendrene	1.32
27.601	Unknown	1.54
27.874	Copaene	4.23
28.004	α-Curcumene	1.74
28.075	β-Selinene	8.16
28.303	(+)-Epi-bicyclosesquiphellandrene	1.64
28.418	Isoledene	1.62
28.664	α-Muurolene	1.73
28.813	*cis*-α-Bisabolene	0.53
29.007	β-Bisabolene	0.71
29.167	α-Cedrene	2.90

TABLE I. Continued

t / min	MS + t_{ret} identification	Content, %
29.242	Unknown	1.23
29.597	δ-Cadinene	17.19
29.917	1,2,3,4,4a,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene	1.29
30.318	α-Calacorene	4.97
30.393	Unknown	1.87
33.562	Cadina-1,4-diene	3.39
34.083	Torreyol	3.91
34.243	γ-Muurolene	tr
34.523	τ-Cadinol	3.12
35.187	γ-Curcumene	3.18
35.256	1,6-Dimethyl-4-(1-methylethyl)naphthalene	4.91

According to the results given in Table II, the essential oil of *T. montanum* had great antibacterial activity against all the investigated microorganisms. The diameters of the growth inhibition zone ranged from 16 to 29 mm (including the 6 mm diameter of the disc) with the highest inhibition zone values observed against *K. pneumoniae* (29 mm). The largest level of resistance was shown by *A. tumefaciens* and *E. carotowora* (an inhibition zone of 16 mm). The essential oil showed similar activity on both Gram-positive and Gram-negative bacteria.

TABLE II. Antimicrobial susceptibility pattern of the essential oil (10 µl/disc) and methanol extract (300 µg ml^{-1}) of *T. montanum*

Microorganism	Inhibition zone diameter^{a,b}, mm		Antimicrobial agent
	Essential oil	Methanol extract	Amracin (300 µg ml^{-1})
Gram(+)			
Bacillus mycoides	25 ± 0.5	19± 0.5	34 ± 0.5
Bacillus subtilis	26 ± 0.5	14± 0.3	29 ± 0.5
Staphylococcus aureus	10 ± 0.3	8 ± 0.3	30 ± 0.5
Gram(–)			
Agrobacterium tumefaciens	16 ± 0.4	13 ± 0.5	32 ± 0.5
Azotobacter chlorococcum	24 ± 0.5	28 ± 0.5	34 ± 0.5
Enterobacter cloaceae	24 ± 0.5	16 ± 0.3	36 ± 0.5
Erwinia carotovora	18 ± 0.3	19 ± 0.5	31 ± 0.5
Klebsiella pneumoniae	29 ± 0.5	22 ± 0.5	35 ± 0.7
Proteus sp.	–	–	11± 0.5
Pseudomonas aeruginosa	–	–	17 ± 0.3
Pseudomonas glycinea	20 ± 0.5	16 ± 0.5	35 ± 0.7
Pseudomonas fluorescens	22 ± 0.5	12 ± 0.3	42 ± 0.7
Pseudomonas phaseolicola	23 ± 0.5	19 ± 0.5	35 ± 0.5

[a]Mean value ± *SD*, n = 3 (the zone of inhibition, in mm, includes the disc of 6 mm diameter); [b]Solvent controls (methanol) were negative

In general, the essential oil showed better activities than the methanol extract. The methanolic extract showed strong antibacterial activity against the bacteria *A. chlorococcum,* the inhibition zone was 28 mm. Both the 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 lower activity than the essential oil.

CONCLUSIONS

According to the obtained results, it is possible to conclude that *Teucrium montanum* had a strong and broad spectrum of antibacterial activity. To the best of our knowledge, this is the first study to provide data on the evaluation of the essential oil of *T. montanum* against bacteria.

The antibacterial activities of the essential oil and methanol extract reported here could be associated with the presence δ-cadinene (17.19 %) and β-selinene (8.16 %). The compounds present in oil indicated a preference of *T. monanum* to synthesize sesquiterpenes.

Based on the obtained results, *Teucrium montanum* essential oil could be used as a preservative in food products, to protect them from microbial spoilage.

ИЗВОД

ХЕМИЈСКИ САСТАВ ЕТАРСКОГ УЉА И АНТИБАКТЕРИЈСКА АКТИВНОСТ ЕТАРСКОГ УЉА И МЕТАНОЛСКОГ ЕКСТРАКТА *Teucrium montanum*

НЕНАД ВУКОВИЋ, ТАЊА МИЛОШЕВИЋ, СЛОБОДАН СУКДОЛАК и СЛАВИЦА СОЛУЈИЋ

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У овом раду је представљено испитивање хемијског састава етарског уља и антибактеријска активност етарског уља и метанолског екстракта *Teucrium montanum*. У највећем проценту етарско уље садржи сесквитерпенске угљоводонике. Забележена је јака антибактеријска активност етарског уља према испитиваним бактеријама. Генерално гледано, етарско уље показује већу активност од метанолског екстракта.

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