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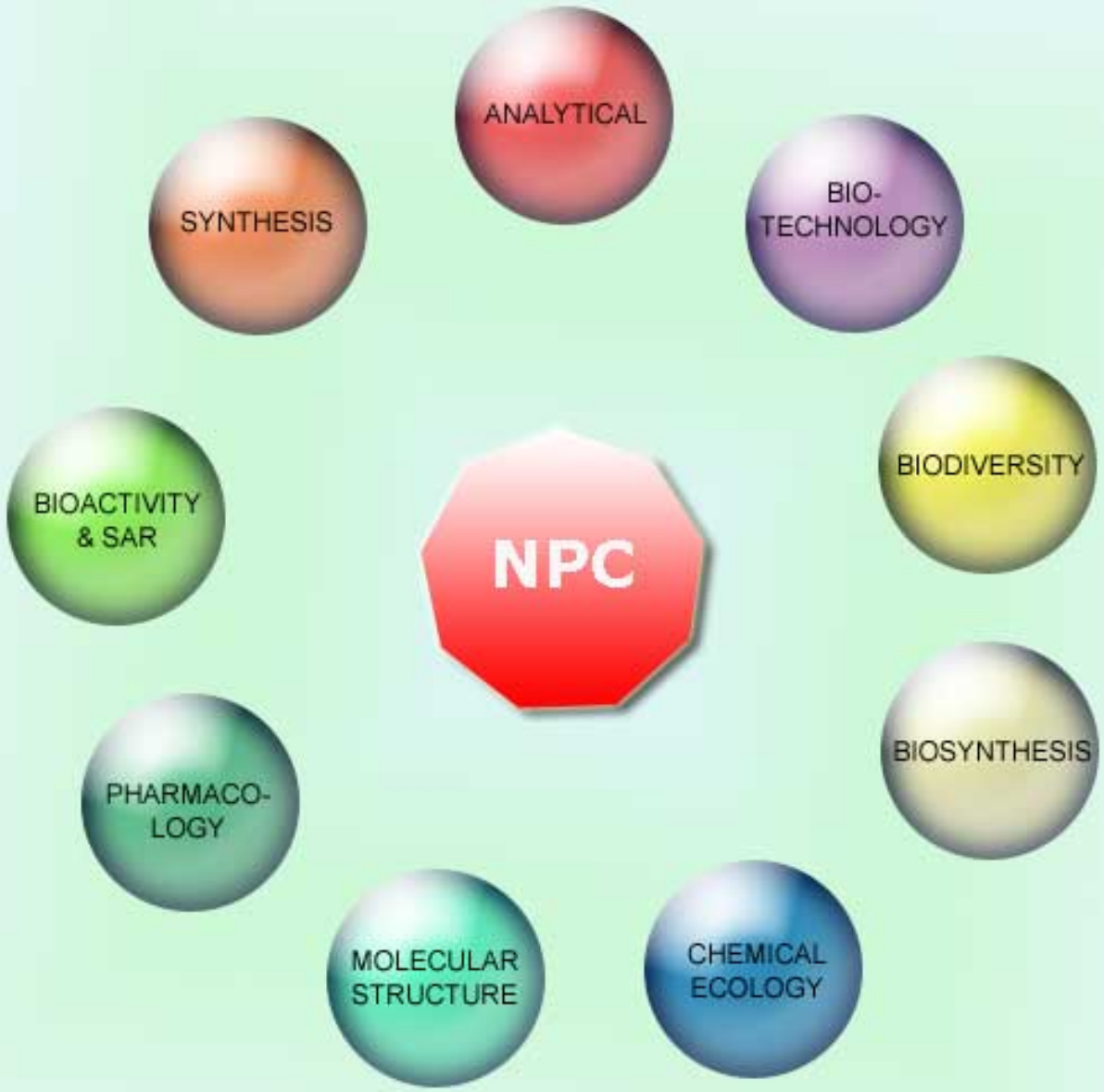

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Chemical Composition and Antimicrobial Activity of the Essential Oil from *Chaerophyllum aureum* L. (Apiaceae)

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The essential oils of the aerial parts and fruits of *Chaerophyllum aureum* L., collected from two mountains in Serbia, were analyzed by GC and GC/MS. Sabinene (18.5-31.6%), *p*-cymene (7.9-25.4%) and limonene (1.9-10.9%) were characterized as the main constituents. The oils were tested against six bacterial strains and one strain of yeast, *Candida albicans.* The highest antimicrobial activity was observed against the Gram-positive bacteria *Staphylococcus aureus*, *S. epidermidis* and *Micrococcus luteus*, while of the Gram-negative strains, *Escherichia coli* was the most sensitive.

Keywords: *Chaerophyllum aureum*, Apiaceae, essential oil composition, sabinene, *p*-cymene, limonene, antimicrobial activity.

The genus *Chaerophyllum* L. (Apiaceae) has about 40 species, which occur commonly throughout Europe, Asia and north America. *C. aureum* L., is a robust, perennial herb up to 150 cm in height, which grows in the mountainous to sub-alpine regions of central and southern Europe [1]. The plants of this genus contain essential oil in the secretory canals in all vegetative and reproductive organs.

The composition of the essential oil from the flowering aerial parts of some *Chaerophyllum* species has been reported [2-6]. In the oil of *C. macropodum* Boiss., the most dominant compounds were *α*-pinene (23.0%), β-pinene (17.3%) and fenchyl acetate (13.8%), whereas in the oil of *C. crinitum* Boiss., fenchyl acetate was absent and (*E*)-β-ocimene (50.5%) was the most prominent [2], which was also the case with the oil of *C. macrospermum* (55.9%) [3]. The main components of the oil of *C. prescottii* DC. were (*E*)-β-ocimene (35.6%), (*Z*)-β-ocimene (19.3%) and γ-terpinene (18.8%) [4], and in the oil of *C. byzantinum* Boiss. sabinene (30.0%), *p*-cymene-8-ol (16%) and terpinolene (11.5%) [5]. Previous investigation of the oils of the aerial parts and fruits of *C. coloratum* L., an endemic species of the Balkan Peninsula, revealed that the major compound was (*E*)-β-farnesene (68.5-79.2%) [6].

The antibacterial activity of the essential oils of *Chaerophyllum* species has not investigated, but that of *C. byzantinum* was reported to be active against *Candida* spp. [5].

The present study was carried out in order to determine the quantitity, chemical composition and antimicrobial activity of the essential oil of *C. aureum,* which have not been previously reported.

Table 1: Composition of the essential oils of *Chaerophyllum aureum*.

Compound	RI	Sample A (%)	Sample B (%)	Sample C (%)
α-Thujene	930	0.7	0.6	0.5
α-Pinene	935	3.8	4.4	5.0
Camphene	950	tr	tr	tr
Sabinene	970	31.6	18.5	18.7
β-Pinene	975	1.5	4.0	7.7
Myrcene	986	2.8	3.3	2.4
n-Octanal	995	-	-	0.3
α-Terpinene	1014	tr	tr	tr
p-Cymene	1020	7.9	25.4	25.3
Limonene	1024	1.9	10.1	10.9
β-Phellandrene	1025	tr	tr	tr
(*Z*)-β-Ocimene	1032	tr	0.7	0.6
(*E*)-β-Ocimene	1045	tr	3.4	3.5
γ-Terpinene	1055	tr	3.2	3.4
cis-Sabinene hydrate	1065	2.5	0.3	0.2
Terpinolene	1085	tr	3.5	3.4
p-Cymenene	1086	0.6	0.5	0.4
trans-Sabinene hydrate	1093	1.4	0.3	0.2
Linalool	1092	tr	tr	tr
cis-*p*-Menth-2-en-1-ol	1117	0.6	0.4	tr
α-Campholenal	1120	tr	tr	tr
Pinocarvone	1160	tr	tr	tr
Terpinen-4-ol	1171	8.6	4.0	3.4
p-Cymen-8-ol	1177	2.2	3.2	2.0
α-Terpineol	1185	0.5	tr	0.2
Myrtenol	1190	0.9	0.5	0.3
Myrtenal	1191	-	0.4	0.2
cis-Piperitol	1192	-	tr	tr
trans-Piperitol	1203	tr	tr	tr
trans-Carveol	1213	tr	0.9	0.2
cis-Carveol	1225	-	tr	tr
Thymol, methyl ether	1230	-	tr	tr
Cumin aldehyde	1237	tr	tr	tr
Carvone	1239	-	0.9	Tr
α-Copaene	1370	tr	tr	tr
β-Bourbonene	1383	-	tr	tr
β-Elemene	1385	-	0.4	0.5
(*Z*)-Jasmone	1388	tr	-	-
(*E*)-Caryophyllene	1414	0.9	0.6	0.7
α-*trans*-Bergamotene	1430	tr	-	-
α-Humulene	1450	3.9	0.3	0.8
(*E*)-*β*-Farnesene	1451	1.1	0.7	2.4
γ-Muurolene	1474	tr	1.2	2.2
ar-Curumene	1476	1.9	tr	tr
(*E*)-*β*-Ionone	1482	tr	tr	tr
Phenyl ethyl 3-methyl butanoate	1485	0.4	tr	-
α-Muurolene	1495	0.6	tr	tr
β-Bisabolene	1500	0.6	tr	tr
γ-Cadinene	1509	0.5	tr	tr
δ-Cadinene	1517	1.5	tr	0.2
α-Cadinene	1534	tr	-	-
α-Calacorene	1540	tr	tr	-
(*E*)-Nerolidol	1557	tr	tr	tr
Spathulenol	1571	4.5	3.4	0.9
Caryophyllene oxide	1577	1.8	1.0	0.6
Salvial-4(14)-en-1-ene	1589	-	tr	tr
Humulene epoxide II	1602	4.2	0.4	0.4
Isospathulenol	1630	tr	0.4	0.3
epi-α-Cadinol	1634	tr	tr	tr
epi-α-Muurolol	1636	1.9	tr	tr
α-Muurolol	1640	tr	-	-
α-Cadinol	1649	3.5	0.1	0.7
cis-Calamenen-10-ol	1655	tr	-	-
trans-Calamenen-10-ol	1663	tr	-	-
Oplopanone	1734	2.2	tr	tr
Neophytadiene	1837	0.7	tr	tr
Hexahydrofarnesyl acetone	1842	tr	tr	tr
Total		**97.7**	**97.0**	**98.5**
Grouped components				
Monoterpene hydrocarbons		50.8	77.6	81.8
Oxygenated monoterpenes		16.7	10.9	6.7
Sesquiterpene hydrocarbons		11.0	3.2	6.8
Oxygenated sesquiterpenes		18.1	5.3	2.9
Others		1.1	-	0.3

RI, Retention Indices relative to C_9-C_{23} *n*-alkanes on the HP 5MS; %, Relative percentage obtained from peak area; tr, trace (< 0.1%).
Sample A, aerial parts from Suva Mountain; Sample B, aerial parts from Kopaonik mountain; Sample C, fruits from Kopaonik mountain

The oil content of the aerial parts of *C. aureum* from both localities was 0.15% (v/w) and of the fruits 0.3% (v/w). The chemical composition of the oils is given in Table 1. In all samples of *C. aureum* oil over fifty compounds were identified, representing 97.7% of sample A, 97.0% of sample B and 98.5% of sample C. The oils were very similar regarding their qualitative pattern, particulary the oils of the aerial parts and fruits from the same collection. In all samples, the most dominant components were monoterpene hydrocarbons (50.8 -81.8%). The main compounds were sabinene (18.5-31.6%), *p*-cymene (7.9-25.4%) and limonene (1.9-10.9%). Some quantitative differences in the main constituents of the oils were observed. The oil from the aerial parts of the plant material collected from Suva Mountain (sample A) differed from the others in its lower content of limonene and *p*-cymene. Sabinene was also reported as the most dominant constituent of the oil from the flowering aerial parts of *C. byzantinum* [5].

The results of the antimicrobial activity of *C. aureum* essential oils are presented in Table 2. The investigated oils showed varying degrees of antimicrobial activity against the tested bacteria, with the exception of *Pseudomonas aeruginosa*, which seemed to be resistant. It is well known that *P. aeruginosa* has a high level of intrinsic resistance to virtually all known antimicrobials and antibiotics due to a very restrictive outer membrane barrier, highly resistant even to synthetic drugs. The highest antimicrobial activity was observed against the Gram-positive bacteria *S. aureus, S. epidermidis* and *M. luteus*, while among the Gram-negative strains, *E. coli* was the most sensitive.

The mechanisms by which essential oils can inhibit microorganisms involve different modes of action, but in part may be due to their hydrophobicity. As a result, they get partitioned into the lipid bilayer of the

Table 2: Antimicrobial activity of the essential oils of *Chaerophyllum aureum*.

Microorganisms	Inhibition zone diameter (mm)										
	Sample A		Sample B		Sample C		Control[b]	AP	AK	BI	NY
	2%	4%	2%	4%	2%[a]	4%[a]					
Gram-positive bacteria											
Staphylococcus aureus (ATCC 25923)	12.7±1.2	14.0±0.0	NA	12.5±2.8	19.0±0.8	19.7±1.9	NA	35.0±7.1	26.5±2.1	NT	NT
Staphylococcus epidermidis (ATCC12228)	10.5±1.0	15.5±1.0	10.0±0.0	19.6±0.6	12.3±0.5	14.5±3.4	10.0±0.0	NT	NT	NT	NT
Micrococcus luteus (ATCC10240)	18.8±2.5	16.3±0.9	12.9±2.0	22.3±2.6	11.3±1.25	16.5±2.4	NA	33.0±0.0	NT	16.75±1.2	NT
Gram-negative bacteria											
Escherichia coli (ATCC 25922)	15.3±0.5	15.0±0.0	15.5±0.5	17.0±1.4	14.4±4.0	17.5±1.0	15.0±0.0	20.5±0.7	20.0±0.0	NT	NT
Pseudomonas aeruginosa (ATCC27853)	NA	NA	NA	NA	11.8±0.5	14.2±1.5	NA	NT	27.5±3.5	NT	NT
Klebsiella pneumoniae (NCIMB 9111)	10.5±1.0	15.5±1.0	15.0±0.0	15.0±0.8	NA	14.8±1.6	NA	NT	NT	NT	NT
Fungi											
Candida albicans (ATCC10259)	NA	12.4±1.5	NA	NA	NA	NA	NA	NT	NT	NT	20.0±0.0

[a]dilution of essential oil in absolute ethanol, v/v; [b]absolute ethanol; NT, not tested; NA, not active; AP, ampicillin (10 μg/disc); AK, amikacin (30 μg/disc); BI, bacitracin (250 units/disc); neomycin (3500 units/disc); NY, nystatin (100 units/discs);
Sample A, aerial parts from Suva Mountain; Sample B, aerial parts from Kopaonik mountain
Sample C, fruits from Kopaonik mountain

cell membrane, rendering it more permeable, leading to leakage of vital cell contents [7-9]. Impairment of bacterial enzyme systems may also be a potential mechanism of antimicrobial action [10].

Experimental

Plant material: Plant material was collected from two localities in Serbia: aerial parts (sample A) in full flowering on Suva Mountain at an altitude of 800 m, in June 2005; aerial parts (sample B) and fruits (sample C) on Kopaonik mountain at an altitude of 1450 m, in August 2005. Voucher specimens were deposited at the Herbarium of the Institute of Botany, Faculty of Pharmacy, University of Belgrade (HFF 1316 and 1317).

Isolation of essential oil: Semi-crushed air-dried plant material was subjected to hydrodistillation for 3 h using a Clevenger-type apparatus.

Gas chromatography: GC analysis was carried out using a SRI 8610C GC-FID system, equipped with a DB-5 capillary column (30 m × 0.32 mm; film thickness 0.25 μm) and connected to a FID detector. The injector and detector temperature was 280°C. The carrier gas was He, at a flow rate of 1.2 mL/min. The thermal program was 60°C to 280°C at a rate of 3°C/min. Two replicates of the sample were processed in the same way.

Gas chromatography-mass spectrometry: GC-MS analysis was performed using a Hewlett Packard 6890-5973 GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (200°C). The transfer line temperature was 250°C. Helium was used as carrier gas (1 mL/min) and the capillary column was a HP 5MS (30 m x 0.25 mm; film thickness 0.25 μm). The temperature program was the same as that used for the GC analysis; split ratio 1:10. The injected volume was 1.0 μL.

Identification of components: The identification of the compounds was based on comparison of their retention indices (RI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the NIST/NBS, Wiley libraries and literature [11]. The linear retention indices (RI) were determined in relation to a homologous series of *n*-alkanes (C_9-C_{23}) under the same operating conditions [12].

Antimicrobial activity: Antimicrobial activity of the essential oils of *C. aureum* was tested against *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Micrococcus luteus*

(ATCC 10240), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (NCIMB 9111) and one strain of yeast, *Candida albicans* (ATCC 10259), by the agar diffusion method [13].

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