SHORT REPORT

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Composition and Antimicrobial Activity of the Essential Oils from Different Parts of *Cachrys cristata* DC. from Greece

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Abstract: Fresh leaves, stems and roots of *Cachrys cristata*, were subjected to hydrodistillation and the oils obtained were analyzed by means of GC and GC-MS. Sixty eight constituents were in total identified, with (*Z*)-β-ocimene (44.2% and 30.5%) dominating in leaves and stems oils, while the major metabolite of the roots was found to be *p*-tolualdehyde (39.6%), followed by (*Z*)-β-ocimene (15.2%). The microbial growth inhibitory properties of the essential oils were determined using the broth microdilution method against seven laboratory strains of bacteria - Gram positive: *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis* and Gram negative: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and two strains of the yeast *Candida albicans*. The essential oils exhibited considerable activity against certain strains of the microorganisms tested, with the leaves oil demonstrating higher antimicrobial activity among the tested samples.

Keywords: Antimicrobial activity; chemical composition; essential oil; *Cachrys cristata*.

1. Plant Source

The genus *Cachrys* (Umbelliferae) is distributed in Eurasia and the Mediterranean area, comprising 8 species in Europe. *Cachrys cristata* DC. (syn. *Hippomarathrum cristatum* DC. Boiss) is a perennial herb widely distributed in South Italy, in the south of Balkan Peninsula and across the Aegean [1].

The plant material was collected from Mt. Parnassos (Viotia Prefecture) in May 2008 during the flowering stage. Voucher specimen (OT-101) has been identified by Dr Theophanis Constantinidis (Department of Ecology and Systematics, University of Athens) and has been kept at the Herbarium of the University of Athens (ATHU).

2. Previous Studies

Coumarins and furanocoumarins have been isolated from the roots and fruits of *Cachrys ferulacea* and from the aerial parts of *C. libanotis* and *C. sicula*, respectively [2]. Additionally *Cachrys* ftruits are rich in psoralene derivatives [2]. Flavonoids have been also reported: quercetin has been isolated from the leaves of *C. libanotis*, *C. trifida* and *C. ferulacea*; moreover kaempferol has been identified from the latter [3]. The fatty acid composition of *Cachrys* seed oils has been reported [4].

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Several reports deal with the volatile constituents of *Cachrys* species. Monoterpenes and sesquiterpenes appear in high percentages also depending -among other factors- on the plant part analyzed [5-13]. *Cachrys cristata* essential oil from Serbia was found to be rich in sesquiterpenes [14].

3. Present Study

Fresh leaves, stems and roots of *Cachrys cristata* were cut in small pieces and subjected independently to hydrodistillation for 3 hours using a modified Clevenger apparatus. The oils were obtained using n-pentane as collecting solvent, and subsequently they were dried over anhydrous sodium sulphate. After filtration the samples were stored under N_2 atmosphere in amber vials at 4^{o}C until analysis.

GC/MS: Analysis of the essential oils was performed using a Hewlett Packard (Hewlett Packard GmbH, Waldbronn, Germany) model 5973-6890 GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (200^{o}C). The transfer line temperature was 250^{o}C. Helium was used as carrier gas (1 mL/min) and the capillary column used was HP 5MS (30 m × 0.25 mm; film thickness 0.25 μm; Agilent, Palo Alto, CA, USA). The temperature program was the same with that used for the GC analysis; split ratio 1:10. The injected volume was 1 μL of diluted essential oil in *n*-pentane (10% v/v). Total scan time 83.33 min. Acquisition mass range 40-400 amu. 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 (purchased from the Sigma-Aldrich Co, (Sigma-Aldrich, Buchs SG, Switzerland) and/or the NIST/NBS, Wiley libraries (available through Hewlett Packard) and the literature [15].

GC/FID: GC analysis of the essential oils was carried out using a SRI (Brooks, Hatfield, PA, USA) 8610C GC-FID system, equipped with DB-5 capillary column (30 m x 0.32 mm; film thickness 0.25 μm; J & W, CA, USA) and connected to a FID detector. The injector and detector temperature was 280^{o}C. The carrier gas was He, at flow rate of 1.2 mL/min. The thermal program was 60 - 280^{o}C at a rate of 3^{o}C/min; split ratio 1:10. Two replicates of each oil were processed in the same way. The injected volume was 1μL of diluted essential oil in *n*-pentane (10% v/v). The integration of the peaks was calculated according to the area % as reported from the PeakSimple software.

Optical rotations of the oils: Optical rotations of the oils were recorded with a Perkin-Elmer Polarimeter 341 (UV-Vis).

Antibacterial-Antifungal activity: The antimicrobial activity was evaluated using seven different laboratory control strains of bacteria - Gram positive: *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis* and Gram negative: *Escherichia coli*, *Klebsiella pneumoniae* , *Pseudomonas aeruginosa* , and two strains of yeast: *Candida albicans*. Microorganisms were provided by the Institute of Immunology and Virology, Torlak, Belgrade. Active cultures for experiments were prepared by transferring a loopful of cells from the stock into tubes that contained 10 mL of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi. After incubation for 24 h at 37°C and 25°C respectively, the cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose broth in order to achieve optical densities corresponding to $4.3x10^6$ colony forming units (cfu mL^{-1}) for bacteria and $1.2x10^7$ cfu mL^{-1} for fungi which was used as inoculum.

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to National Committee for Clinical Laboratory Standards (NCCLS) [16]. The inoculum of the bacterial strain was prepared from overnight broth culture and suspension was adjusted to 0.5 McFarland standard turbidity. The tested oils were dissolved in 1% dimethylsulphoxide (DMSO) and then diluted to the highest concentration. Two-fold serial concentrations of the essential oil were prepared in a 96-well microtiter plate.

In the tests, triphenyl tetrazolium chloride (TTC) (Aldrich Chemical Company Inc. USA) was also added to the culture medium as a growth indicator. The final concentration of TTC after inoculation was 0.05%. The microbial growth was determined by absorbance at 620 nm using the universal microplate reader (ThermoLabsystems, Multiskan EX, Software for Multiscan ver.2.6.) after incubation at 37°C for 24 h for bacteria, and at 26°C for 48 h for fungi. The MIC is defined as the

lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. All determinations were performed in triplicate and two positive growth controls were included. The MICs of the reference antibiotics were determined in parallel experiments.

After detailed analyses of GC and GC-MS data sixty eight constituents were totally identified (Table 1). The essential oils of both leaves and stems were characterized by the exceptionally high abundance of monoterpenes (80.7% and 83.2%, respectively) with (*Z*)-β-ocimene (44.2% and 30.5%) being the major metabolite of the oil, followed by δ-3-carene (8.7%) and *p*-cymene (10.7%), respectively. The percentage of sesquiterpenes was 18.5% in leaves, while in stems it was a little lower (11.7%), with trans-caryophyllene (5.5% and 4.8%, respectively) identified as the main component. In the essential oil of the roots *p*-tolualdehyde, a metabolite that has not been detected in leaves and stems was found in a high percentage (39.6%). The oil of roots was also rich in monoterpenes (50.8%), with (*Z*)-β-ocimene (15.2%) being the main metabolite.

Contrary to our results the essential oil from the aerial parts and fruits of *C. cristata* of Serbian origin was characterized by an extremely high percentage of sesquiterpenes (78.6%, 85.0% respectively) [14]. Monoterpenes were the main class of components in the essential oils from different plant parts of *C. ferulacea* [11, 12]. Pala–Paul et al. reported on the volatile constituents of *C. trifida* from Spain; the main metabolites of the leaves and stems essential oils were (*Z*)-β-ocimene and (*E*)-β-ocimene, which is partly in accordance with our results [7]. Also they have investigated the root essential oil, in which terpinolene, γ-terpinene and *p*-cymene appeared to be the most abundant constituents, while *p*-tolualdehyde –the major metabolite of our analysis- was not detected at all [7]. Essential oil of *C. alpina* fruits has been reported to have as main constituents α-humulene (33.1%), *p*-cymene (9.3%), α-phellandrene (9.1%), germacrene D (8.2%) and α-pinene (6.3%) [6]. Sefidkon and Shaabani studies on the essential oil composition of a closely related species *Hippomarathrum microcarpum* from Iran showed that the leaves oil was characterized by a high percentage of sesquiterpenes with trans-caryophyllene being the major metabolite [8]. In the fruit oil of *Hippomarathrum cristatum* of Turkish origin, hexadecanoic acid, nonacosane, germacrene D and myristicin were found to be the major metabolites [5], whereas in the studied oils only germacrene D was present.

Table 1. Chemical composition (%) of *Cachrys cristata* leaves, stems and roots essential oils.

RI[a]	Compound[b]	Leaf	Stem	Root
	Monoterpene hydrocarbons			
925	α-thujene	t	t	0.3
931	α-pinene	3.7	7.2	2.7
950	camphene	t	1.5	t
970	sabinene	3.7	3.5	2.6
986	myrcene	3.0	3.8	2.6
997	α-phellandrene	t	t	11.7
999	δ-3-carene	8.7	8.8	2.9
1020	*p*-cymene	2.7	10.7	t
1032	(*Z*)-β-ocimene	44.2	30.5	15.2
1045	(*E*)-β-ocimene	2.0	1.4	7.9
1055	γ-terpinene	6.0	6.9	1.5
1079	*p*-cymenene	-	-	t
1084	terpinolene	5.2	6.7	-
1105	1,3,8-*p*-menthatriene	t	-	t
1127	*allo*-ocimene	1.5	1.2	-
	∑	**80.7**	**82.2**	**47.4**
	Oxygenated monoterpenes			
1116	dehydro-sabina ketone	t	-	-
1127	(*Z*)-epoxy-ocimene	t	t	-
1132	(*E*)-epoxy-ocimene	-	0.4	-
1172	terpinen-4-ol	t	0.6	-
1177	*p*-cymen-8-ol	t	t	-
1180	cryptone	-	-	0.4
1230	thymol methyl ether	t	t	-
1240	carvacrol methyl ether	t	t	-
1252	carvenone	t	-	-
1279	bornyl acetate	t	t	0.3
1282	*trans*-sabinyl acetate	-	-	2.4
	∑	**t**	**1.0**	**3.1**

	Sesquiterpene hydrocarbons			
1328	δ-elemene	t	-	-
1370	α-copaene	t	t	-
1372	daucene	t	t	-
1378	β-cubebene	t	t	-
1381	β-elemene	t	t	-
1404	β-caryophyllene	5.5	4.8	-
1422	γ-elemene	t	t	-
1426	aromadendrene	t	-	-
1440	α-humulene	0.8	0.6	-
1442	(*E*)-β-farnesene	t	t	t
1463	γ-muurolene	-	t	-
1470	germacrene D	2.4	1.8	-
1474	β-selinene	0.5	0.5	-
1483	α-selinene	-	-	0.3
1485	bicyclogermacrene	2.0	1.3	-
1486	α-muurolene	-	-	t
1492	α-cuprenene	t	-	-
1493	(*E*,*E*)-α-farnesene	t	-	-
1494	β-bisabolene	t	-	1.1
1500	γ-cadinene	t	-	-
1508	δ-cadinene	1.3	0.9	-
1509	β-sesquiphellandrene	t	-	-
1515	zonarene	0.8	0.8	-
1532	selina 3,7(11)-diene	0.8	1.0	-
1547	germacrene B	t	-	-
	∑	**14.1**	**11.7**	**1.3**
	Oxygenated sesquiterpenes			
1412	2,5-dimethoxy-*p*-cymene	-	-	0.3
1458	7-*epi*-dehydrosesquicineole	t	t	-
1485	4-*epi*-*cis*-dihydro-agarofuran	-	-	0.7
1518	kessane	-	-	1.4
1535	elemol	-	-	t
1548	(*E*)-nerolidol	-	-	-
1563	spathulenol	t	t	-
1568	caryophyllene oxide	t	0.5	-
1577	viridiflorol	-	-	-
1625	*epi*-α-muurolol	-	-	-
1637	α-eudesmol	-	-	-
1671	α-bisabolol	1.9	1.2	-
1808	β-vetivone	2.5	3.2	-
	∑	**4.4**	**4.9**	**2.4**
	Other components			
895	*n*-nonane	-	-	0.5
1072	*p*-tolualdehyde	-	-	39.6
1491	(2*E*)-undecenol acetate	-	-	0.4
2100	*n*-heneicosane	t	-	-
	∑	**t**	**t**	**40.5**
	$[\alpha]_D^{20}$=	+1.35 ^{o} (*c*. 9.47. $CHCl_3$)	+8.06 ^{o} (*c*. 0.6. $CHCl_3$)	+7.35^o (*c*. 0.6. $CHCl_3$)
	Total	**99.2**	**99.8**	**94.8**

[a]RI: Retention indices on HP-5 MS column relative to C_9-C_{23} n-alkanes.
[b]Constituents listed in order of elution from a HP-5 MS column.
t: trace (<0.1%), -: not detected

The essential oil of the leaves (L) exhibited in general higher antibacterial activity in comparison to samples S and R (Table 2). The most sensitive were Gram (+) bacteria *Staphylococcus aureus*, *Micrococcus luteus* and *Staphylococcus epidermidis*.

A considerable activity was demonstrated by the stems essential oil (S) against *Staphylococcus aureus* and a moderate one against *Micrococcus luteus* and *Staphylococcus epidermidis*, while it was moderately effective against one strain of *Candida albicans*.

The essential oil of the roots showed a relatively good activity against *Micrococcus luteus*, while it exhibited a moderate one against *Staphylococcus epidermidis*.

Table 2. Antimicrobial activity of *Cachrys cristata* leaves, stems and roots essential oils.

	Microorganism	Minimum inhibitory concentration MIC (μg mL^{-1})					
		Leaf	**Stem**	**Root**	**Amikacin**	**Ampicillin**	**Nystatin**
1.	*Staphylococcus aureus* ATCC 25923	6.78	14.71	>124	2.0	1.0	n.t.
2.	*S. epidermidis* ATCC 12228	54.25	58.83	62.00	n.t.	n.t.	n.t.
3.	*Micrococcus luteus* ATCC 9341	6.78	29.42	15.50	n.t.	n.t.	n.t.
4.	*Enterococcus faecalis* ATCC 29212	>108.50	>117.67	>124.0	2.4	n.t.	n.t.
5.	*Escherichia coli* ATCC 25922	>108.50	>117.67	>124.0	8.6	4.4	n.t.
6.	*Klebsiella pneumoniae* NCIMB 9111	>108.50	>117.67	>124.0	6.4	n.t.	n.t.
7.	*Pseudomonas aeruginosa* ATCC 27853	>108.50	>117.67	>124.0	2.8	n.t.	n.t.
8.	*Candida albicans* ATCC 10259	>108.50	29.42	>124.0	n.t.	n.t.	3.8
9.	*Candida albicans* ATCC 24433	>108.50	>117.67	>124.0	n.t.	n.t.	6.2

n.t. not tested

References

[1] T. G. Tutin (1968). *Cachrys* L., in Flora Europaea, T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S.M. Walters and D.A. Webb, Cambridge University Press, Cambridge, **2**, pp. 343-344.

[2] R. Hegnauer (1990). *Chemotaxonomie der Pflanzen*, Birkhäuser Verlag, Basel, **IX**, p.606, 684 .

[3] R. K. Crowden, J. B. Harborne and H. Heywood (1969). Chemosystematics of the Umbelliferae- A general survey, *Phytochemistry.* **8**, 1963-1984.

[4] M. Öztürk, U. Geçgel, A. Duran, N. Uslu and M. M. Özcan (2014). The fatty acids compositions of several plants seed oils belong to Leguminosae and Umbelliferae families, *Environ. Monit. Assess*. **186**, 2795-2799.

[5] G. Ozek, T. Ozek, K.H.C. Baser, E. Hamzaoglu and A. Duran (2007). Composition of the Essential Oil of *Hippomarathrum cristatum* (DC.) Boiss., *J. Essent. Oil. Res*. **19**, 540-542.

[6] K. H. C. Baser, B. Demirci, E. Akalin and N. Ozhatay (2004). Composition of the Essential Oil of *Cachrys alpina* Bieb., *J. Essent. Oil Res.* **16**, 167-168.

[7] J. Pala-Paul, A. Velasco-Negueruela, M.J. Perez-Alonso, J. Maqueda and J. Sanz (2004). Volatile Oil Constituents from Different Parts of *Cachrys trifida* L., *J. Essent. Oil Res.* **16**, 347-349.

[8] F. Sefidkon and A. Shaabani (2003). Analysis of the Oil of *Hippomarathrum microcarpum* (M.B.) B. Fedtsch. from Iran, *J. Essent. Oil Res.* **15**, 261-262.

[9] J. Pala-Paul, A. Velasco-Negueruela, M.J. Perez-Alonso and J. Sanz (2002). Essential oil composition of the aerial parts of *Cachrys sicula* L. , *Flavour Fragr. J*. **17**, 64-68.

[10] K. H. C. Basser, T. Ozek and Z. Aytac (2000). Essential Oil of *Hippomarathrum boissieri* Reuter et Hausskn, *J. Essent. Oil Res*. **12**, 231-232.

[11] A. Bertoli, L. Pistelli, I. Morelli, G. Spinelli and A. Manunta (1998). Constituents of *Cachrys ferulacea* Oils, *J. Essent. Oil Res*. **10**, 533-536.

[12] F. Sefidkon, M. Khajavi, and B. Malackpour (1998). Analysis of the Oil of *Prangos ferulacea* (L.) Lindl., *J. Essent. Oil Res*. **10**, 81-82.

[13] K. H. C. Baser, T. Ozek, B. Demircakmak, A. Bicakci and H. Malyer (1996). Essential Oil of *Echinophora chrysantha* Freyn et Sint., J. *Essent. Oil Res.* **8**, 433-434.

[14] J. S. Matejić, A. M. Džamić, M.S. Ristić, V. N. Randelović and P. D. Marin (2012). Essential Oil Composition of *Cachrys cristata* – a Rare and Endangered Species in the Flora of Serbia, *Nat. Prod. Commun.* **7**, 235-236.

[15] R. P. Adams (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 3th and 4th edition. Allured Publishing Corporation Carol Stream, Illinois, USA.

[16] National Committee for Clinical Laboratory Standards (NCCLS) (2001). Performance standards for antimicrobial susceptibility testing: Elevent informational supplement. Document M100-S11, Wayne, Pennsylvania