SUPPLEMENTARY MATERIAL

Chemical composition of the essential oil of *Cyanus adscendens* (Bartl.) Soják and *C. orbelicus* (Velen.) Soják growing wild in Bulgaria, and PCA analysis of genus *Cyanus* Mill.

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Chemical composition of the essential oil of *Cyanus adscendens* (Bartl.) Soják and *C. orbelicus* (Velen.) Soják growing wild in Bulgaria, and PCA analysis of genus *Cyanus* Mill.

Cyanus Mill. genus, belonging to the Asteraceae family, includes more than fifty taxa, mainly growing in Central and Southern Europe, North Africa, Asia Minor, and the Caucasus. Previous investigations on *Cyanus* taxa have shown that they are rich source of flavonoids and phenolic compounds but, differently from species of genus *Centaurea*, almost devoid of sesquiterpene lactones.
In the present study, the chemical composition of the essential oils from aerial parts of *Cyanus adscendens* (**CA**) and *C. orbelicus* (**CO**), collected in Bulgaria, and not previously investigated, was evaluated by GC-MS. The main components of **CA** were *α*-bergamotene (31.3%), (*Z*,*Z*,*Z*)-9,12,15-octadecatrien-1-ol (14.5%) and calarenepoxide (11.0%). Caryophyllene oxide (12.0%), together with *α*-cadinol (10.9%) and spathulenol (8.8%), were recognized as the main constituent of *C. orbelicus* EO. Furthermore, a complete review on the composition of all essential oils of the *Cyanus* taxa studied so far has been inserted and cluster analysis (PCA) was carried out.

Keywords: Asteraceae; essential oils; *α*-bergamotene; caryophyllene oxide; calarenepoxide; PCA and HCA analysis

2. Results and discussion

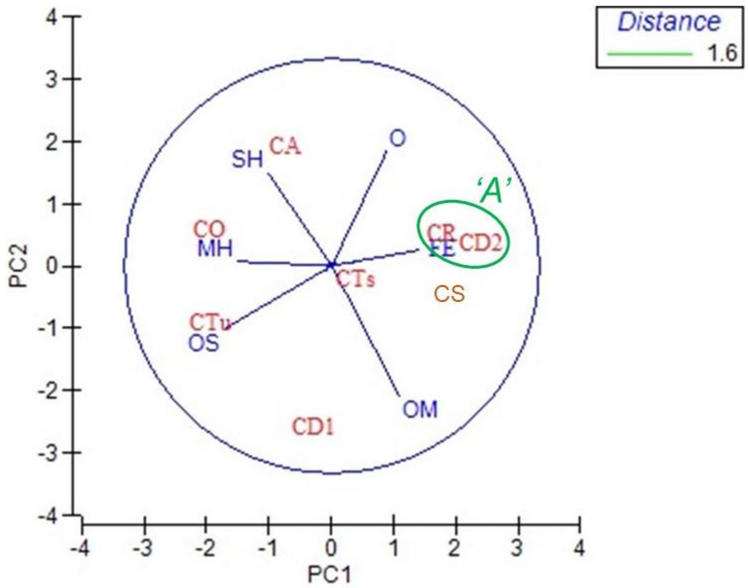

Figure S1. Principal component analysis (PCA) of the essential oils of published plants belonging to the *Cyanus* genus based on the principal classes of compounds: monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpenes hydrocarbons (SH), and oxygenated sesquiterpenes (OS), fatty acids and esters (FE), and others (O). The vectors shown are the eigenvectors of the covariance matrix.

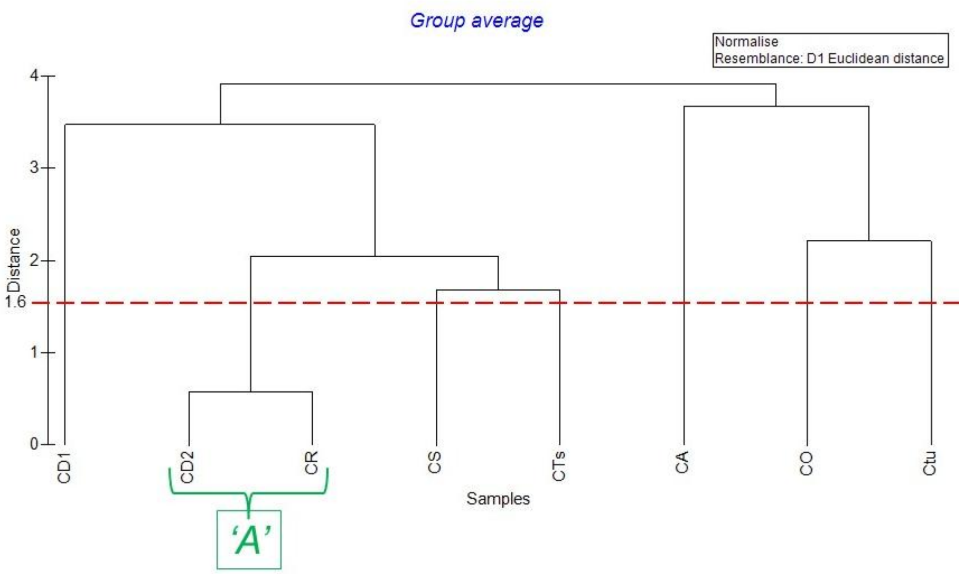

Figure S2. Dendrogram obtained by Hierarchical Cluster Analysis (HCA) based on the Euclidian distances between the essential oils of published plants belonging to the *Cyanus* genus.

Table S1. Chemical composition of essential oil from *Cyanus adscendens* aerial parts.

No.	**Compounds**[a]	**LRI**[b]	**LRI**[c]	**%**[d]
1	Isoledene	1378	1373	0.54
2	Cyperene	1393	1391	1.90
3	Caryophyllene[e]	1415	1419	5.05
4	*β*-Maaliene	1422	1419	0.39
5	*β*-Copaene	1428	1429	1.58
6	*α*-Bergamotene	1431	1433	31.34
7	*β*-*trans*-Farnesene	1437	1439	4.32
8	*α*-Guaiene	1439	1441	0.80
9	*β*-Santalene	1471	1476	4.29
10	Dodecanol[e]	1475	1478	3.18
11	*α*-Curcumene	1485	1484	1.55
12	*β*-Sesquiphellandrene	1521	1527	1.09
13	Spathulenol[e]	1572	1578	2.24
14	Ledene alcohol	1575	1570	0.90
15	Isoaromadendrene epoxide	1578	1581	0.93
16	Caryophyllene oxide	1586	1589	0.52
17	Calarenepoxide	1594	1591	11.03
18	Humulene oxide II	1609	1611	1.51
19	*α*-Cadinol	1659	1663	1.28
20	*α*-*trans*-Bergamotenol	1703	1707	1.20
21	(*Z*,*Z*)-9,12-Octadecadien-1-ol	1875	1869	3.84
22	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrien-1-ol	2047	2058	14.53
	Sesquiterpene Hydrocarbons			**52.85**
	Oxygenated Sesquiterpenes			**19.61**
	Other			**21.55**
	Total			**94.01**

[a] Components listed in order of elution on an DB-5MS column; [b] Linear retention indices on a DB-5MS apolar column; [c] Linear retention indices based on literature (https://webbook.nist.gov/); [d] Percentage amounts of the separated compounds calculated from integration of the peaks (TIC); [e] Co-injection with authentic standards.

Table S2. Chemical composition of essential oil from *Cyanus orbelicus* aerial parts.

No.	Compounds[a]	LRI[b]	LRI[c]	%[d]
1	*o*-Allyltoluene	1008	1004	6.00
2	Limonene[e]	1028	1031	1.40
3	Decanal[e]	1202	1206	1.10
4	Thymol[e]	1297	1299	0.42
5	β-Cubebene	1387	1389	2.27
6	Caryophyllene[e]	1415	1419	1.80
7	Humulene	1458	1456	0.65
8	γ-Muurolene	1469	1475	1.17
9	Aristolene	1485	1486	3.12
10	δ-Cadinene	1519	1519	2.37
11	Spathulenol[e]	1572	1578	8.81
12	Isoaromadendrene epoxide	1578	1581	5.05
13	Caryophyllene oxide	1586	1589	12.02
14	Boronia butenal	1589	1584	2.17
15	5,5-Dimethyl-4-[(1*E*)-3-methyl-1,3-butadienyl]-1-oxaspiro[2.5]octane	1612	-	1.33
16	Ledene oxide	1652	1646	2.42
17	α-Cadinol	1659	1663	10.91
18	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenol	1711	1714	4.50
19	Hexahydrofarnesyl acetone	1831	1827	1.08
20	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrien-1-ol	2047	2058	3.55
21	Tricosane[e]	2300	2300	3.76
22	Tetracosane[e]	2400	2400	6.51
23	Heptacosane[e]	2700	2700	7.19
24	Nonacosane[e]	2900	2900	2.43
	Monoterpene Hydrocarbons			**7.40**
	Oxygenated Monoterpenes			**0.42**
	Sesquiterpene Hydrocarbons			**11.38**
	Oxygenated Sesquiterpenes			**48.29**
	Other			**24.54**
	Total			**92.03**

[a] Components listed in order of elution on an DB-5MS column; [b] Linear retention indices on a DB-5MS apolar column; [c] Linear retention indices based on literature (https://webbook.nist.gov/); [d] Percentage amounts of the separated compounds calculated from integration of the peaks (TIC); [e] Co-injection with authentic standards.

3. Experimental

3.1. Plant material

Aerial parts from twenty individuals, covering about 300 m^2, of *C. adscendens* (**CA**), at full flowering stage, were collected at Ushi village, Bulgaria, (42°31’33” N; 22°33’35” E; 1150 m s/l), in July 2021. Aerial parts from twenty individuals, covering about 300 m^2, of *C. orbelicus* (**CO**), at full flowering stage, were collected at Rila, Mt. at Kalin dum, Bulgaria (42°07’59” N; 23°32’59” E; 2300 m s/l), in July 2021. Samples, leg. & det. by S. Bancheva, have been stored in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM) (Vocher No. SOM-177 583 and SOM-177 584, respectively).

3.2. Essential oil extraction

Air-dried samples were ground in a Waring blender and then subjected to hydrodistillation for 3 h, according to the standard procedure described in European Pharmacopoeia (2020). The oils were dried over anhydrous sodium sulphate and stored in sealed vial under N_2, at -20 °C, ready for the GC and GC-MS analyses; the sample yielded 0.07% and 0.08% of oil (w/w), for **CA** and **CO**, respectively.

3.3. GC and GC-MS analysis

Analysis of essential oil was performed according to the procedure reported by Basile et al. 2022. GC-MS analysis was performed with an Agilent 7000 C GC (Agilent Technologies, Inc., Santa Clara, CA, USA) system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) and coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973 (Agilent Technologies, Inc., Santa Clara, CA, USA). The percentage in Table S1 are calculated with the TIC from MS. The settings were as follows: ionization voltage, 70 eV; electron multiplier energy, 2000 V; transfer line temperature, 295 °C; solvent delay, 4 min. Linear retention indices (LRI) were determined by using retention times of *n*-alkanes (C_8-C_{40}) and the peaks were identified by comparison with mass spectra and by comparison of their retention index with WILEY275, NIST 17, ADAMS, and FFNSC2 libraries.

3.4. Statistical Analysis

Principal component analysis was performed according to the procedure reported by Catinella et al. 2021. The different chemical classes used to describe the composition of individual essential oils were considered as original variables and subjected, after normalization, to cluster analysis (CA) and to principal component analysis (PCA). The statistical analyses were performed using PRIMER 6 (Massey University Eastbourne, Albany, New Zealand) with two principal components (PC) variables, and the number of clusters was determined by using the rescaled distances in the dendrogram, using a cut-off point (Euclidean distance = 1.6) that allows the attainment of consistent clusters. The principal components analysis (PCA) and the hierarchical cluster analysis (HCA) were used to comprehend the similarities among the essential oils in relation to the contents of their chemical constituents. We tested only a cut-off similarity levels (cut-off level 1.6), chosen based on the mean distance between clusters and based on the similarities–differences between the samples belonging to the same cluster. Since the HCA analysis is a function of variables and observations, the highest correspondence between PCA and HCA resulted when we applied a cut-off of 1.6. The statistical analysis of the absence/presence was carried out using the cluster method of the PRIMER 6 software (Massey University Eastbourne, Albany, New Zealand) (Clarke and Gorley 2006).

References

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