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Chemical Composition of Two Endemic Centaurea L. Taxa from Turkey, A Chemotaxonomic Approach

Omer Kilic 1* and Eyüp Bagci²

¹ Bingol University, Technical Science Vocational College, Bingol, Turkey ²Firat University, Science Faculty, Biology Deptartment, Plant Products and Biotechnology Lab., Elazig-Turkey

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Abstract: In this study two endemic Centaurea L. species from Turkey (C. kurdica Reichardt and C. saligna (K.Koch) Wagenitz) which were collected in the similar habitat, have been investigated. The hydro distilled essential oil of aerial parts of C. kurdica and C. saligna were analysed by GC and GC-MS. As a result thirty five and thirty seven components were identified representing 89.0 % and 89.6 % of the oil, respectively. Germacrene D (28.3 %), caryophyllene oxide (10.5 %) and β -caryophyllene (9.5 %) were detected main compounds of *C. kurdica*, however caryophyllene oxide (25.2 %), β-eudesmol (11.5 %) and germacrene D (10.2 %) were detected major constituents of C. saligna. Studied species manufactured many similar constituents in their essential oils that could be verified by the same ecological conditions of their habitat, but also differences were detected that could approve their taxonomic separation. The results have given some clues on the chemotaxonomy of these taxa.

Key words: Centaurea, Chemotaxonomy, Essential oil, Germacrene D, Caryophyllene oxide.

Introduction

Centaurea L. (Asteraceae or Compositae) is included by a very large number of taxa, distributed in particular in central, southwest and east of the Anatolia. Furthermore Centaurea is a polymorphous genus and comprises 400-700 species of annual, biennial and perennial grassy plants, rarely dwarf shrubs predominantly distributed in Europe and Asia¹. Asteraceae represented in Turkey with 140 genus, 1186 species which 446 of are endemic and Centaurea represented in Turkey with 179 native species which 111 of are endemic and it is the richest genera in terms of endemic species with the rate of 62 %². Then new Centaurea taxa were found from Turkey; so with 38 new taxa, Centaurea is represented in Turkey with 217 taxa ³⁻⁵.

Centaurea represented in Turkey with 34 sect. C. saligna belongs to section Cheirolepis (Boiss.) O. Hoffm. and C. kurdica belongs to Cynaroides Boiss. ex Walp section. C. kurdica and C. saligna are endemic species for Turkey and distributed mainly in Eastern Anatolia. They are herbaceous perennial herbs grown in mountain slopes and dry lands. Flower colour is a distinctive character between two species. C. saligna's flowers yellow but C. kudica's are pink, purple or whitish. Taxonomically the genus Centaurea needs more researches, mainly using modern research techniques. The unnatural district of this genus is a very old problem 6 that based on karyological, morphological and palynological diversity 7; main problems that should be solved this genus are: some sections could be proceeded as genera, the exact

^{*}Corresponding authors (Omer Kilic)

E-mail: < omerkilic77@gmail.com >

delimitation of many taxa of some sections should be explained ⁸. The aerial parts of the plant are known as peygamber cicegi, zerdali dikeni, coban kaldiran, timur dikeni in Turkey ⁹. Previous chemical studies on the genus *Centaurea* seem to indicate that the sesquiterpene lactones are the most characteristic constituents and systematically important ¹⁰. Other secondary metabolites present in plants of this genus include triterpenes ¹¹, steroids ¹², hydrocarbons, polyacetylenes, flavonoids ¹³, anthocyanins, lignans ¹⁴, alkaloids ¹⁵ and essential oils *C. behen* L. ¹⁶.

Due to the chemical variability of Centaurea taxa, the purpose of this study is to determine essential oil composition of two Centaurea species, to compare with the genus patterns and to examine potential chemotaxonomic significance infrageneric means. Cluster analysis was performed to the major essential oil compounds from this study and from the literature reviews on the Centaurea taxa essential oils all around world. The studies on the Asteraceae plant group are continuing in our lab. 17-19. This research deals with the chemical composition of two endemic Centaurea species from Turkey; C. kurdica and C. saligna studied fort he first time in this study. We have chosen studied species growing in the similar habitat and with same ecological needs to evaluate if the pedoclimatic circumstances could effect the essential oil composition cause chemical convergence and to provide chemical data that might be helpful in chemotaxonomy Centaurea taxa.

Materials and methods *Plant material*

C. kurdica (Kilic 2650) and *C. saligna* (Kilic 2651) were collected at the full flowering period from plants grown at north part of Örnek village (altitude of 1400-1450 m), Elazig-Keban/Turkey, in July 2010.

Isolation of the essential oil

Aerial parts of the dried plant materials (100 g) were exposed to hydrodistillation using a Clevenger apparatus for three hour.

Gas chromatography

The essential oil of plant samples were analyzed

using HP 6890 GC equipped with and FID detector and used capillary an HP- 5 MS column (30 m \times 0.25 mm i.d., film tickness 0.25 μ m). The column and analyse circumstances were the same as in GC-MS. The percentage of the essential oil composition was calculated from GC-FID peak areas without correction factors.

Gas chromatography-Mass spectrometry

The essential oils were analyzed by GC-MS, using a Hewlett Packard technique. HP-Agilent 5973 N GC-MS system with 6890 GC in Firat University. HP-5 MS column ($30 \text{ m} \times 0.25 \text{ mm}$ i.d., film tickness ($0.25 \mu \text{m}$) was used with helium as the trailer gas. Injector temperature was 250°C, split flow was 1 mL/min. The GC oven temperature was protected at 70°C for 2 min. and programmed to 150°C at a rate of 10°C/min and then kept fixed at 150°C for 15 min to 240°C at a rate of 5°C / min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). MS were taken at 70 eV and a mass range of 35-425.

Supplemantary identification was determined using Wiley and Nist libraries. Hierarchical cluster analysis of twenty six *Centaurea* taxa are seen in Figure 1. The essential oils composition of two *Centaurea* species is showed in Table 1 and the main constituents of *Centaurea* taxa from literature and studied samples is listed in Table 2.

Statistical analysis

The statistical software Cropstat (IRRI 2005) was used to perform the ANOVA and pattern analysis. Standard analyses of variance (Anova) were used to analyze the data obtained.

Results and discussion

The chemical composition essential oil of dried aerial parts of *C. kurdica* and *C. saligna* were analyzed by GC and GC-MS. 35 and 37 compounds were identified in *C. kurdica* and *C. saligna* respectively, accounting from 89.0 % to 89.6 % of the whole oil. The yield of oils are ca. 0.30 and 0.40 mL/100 g respectively. The main compounds of *C. kurdica* were germacrene D (28.3 %), caryophyllene oxide (10.5 %) and β-caryophyllene (9.5 %), while in the *C. saligna* caryophyllene oxide (25.2 %), β -eudesmol (11.5 %) and germacrene D (10.2 %). Total fifty compounds have been determined. C. kurdica and C. saligna oils are characterized by the presence of sesquiterpenes; mainly hydrocarbon derivatives and in small amounts oxygenated ones. Sesquiterpenes accounted in almost all the samples more than 50 % of the whole oils; among the sesquiterpenes, germacrene D, β -caryophyllene, caryophyllene oxide, spathulenol, β -eudesmol were the main compounds and, in small amounts, but shared by two species, bicyclogermacrene, β -cubebene, β bisabolene, α -cadinol, β -selinene and α -humulene. Monoterpenes were always less than 10 %; among them, α -pinene and β -pinene, myrcene, α -phellandrene, p-cymene, α -copaene and limonene were the most constantly reported constituents. Other common compounds were terpenes, alcohols, fatty acids such as hexadecanoic acid and others.

All studied Centaurea species included high concentrations of germacrene D (28.3 - 10.2 %, respectively) and caryophyllene oxide (10.5 - 25.2 %, respectively). C. kurdica had 28.3 % of germacrene D, but its content of β -caryophyllene was smaller (9.5%). C. saligna was described by their lower content of β -caryophyllene and germacrene D (3.3 % and 10.2 %, respectively), but showed high amounts of caryophyllene oxide (25.2 %) (Table 1). Among the sesquiterpenes, caryophyllene oxide was found principal constituents of C. saligna (25.2 %), C. kurdica (10.5 %) (Table 1), this compound also principal constituents of C. helenioides (18.2 %) 20 and C. behen L. (15.9 %)¹⁶. C. kurdica and C. aladaghensis belongs to Cynaroides Boiss. ex Walp section, germacrene D (28.3 %, 22.7 %) and β caryophyllene (9.5 %, 18.3 %) were the principal components of two species respectively. So we can say germacrene D and β -caryophyllene is the chemotaxonomic marker for section Cynaroides. β -eudesmol was found high percentage in *C. saligna* (11.5 %) (Table 1) and *C. cuneifolia* (26.5 %) ²¹ and β -caryophyllene detected as the main compound of *C. kurdica* (9.5 %) (Table 1), *C. hadimensis* (9.8 %) ²² and *C. kotschyi* var. *kotschyi* (12.1 %) ²³. Sesquiterpenes are the main class and among these β -caryophyllene oxide, followed by β -eudesmol and spathulenol (Table 2).

C. hadimensis, C. pseudoscabiosa subsp. pseudoscabiosa, C. kotschyi var. decumbens, C. kotschyi var. kotschyi, C. solstitialis, C. chrysantha, C. mucronifera and C. cineraria subsp. umbrosa contained high concentrations of germacrene D (44.3 %, 36.0 %, 29.4 %, 44.2 %, 61.0 %, 27.4 %, 29.3 % and 22.0 %, respectively) and β-caryophyllene (8.1 %, 9.8 %, 11.2 %, 12.1 %, 4.3 %, 7.3 %, 4.2 % and 8.6 %, respectively). C. cuneifolia and C. euxina have different chemical properties from all the other Centaurea taxa, producing high concentration of hexadecanoic acid (17.6 - 20.3 %) and spathulenol (6.3 - 10.8 %), followed by no percentages of β -caryophyllene and bicyclogermacrene (Table 2). C. pseudoscabiosa subsp. pseudoscabiosa and C. chrysantha belongs to same section (Acrocentron), germacrene D (36.0 - 27.4 %) was the principal components of two taxa respectively. So we can say germacrene D is the chemotaxonomic marker for section Acrocentron. C. saligna, C. kotschyi

Constituents	RRI*	C. kurdica	C. saligna
(E)-2-Hexenal	855	0.5	0.2
2-Heptanone	890	0.5	- 0.2
Heptanal	901	-	0.1
α-Pinene	935	0.8	1.4
Benzaldehyde	962	-	0.2
Sabinene	977	0.1	-
β-Pinene	982	0.5	0.7
2-pentylfuran	990	0.3	-
Santolinatriene	998	-	-

 Table 1. Identified components of Centaurea taxa (%)

table 1. (continued).

Constituents	RRI*	C. kurdica	C. saligna
Octanal	1003	-	0.5
α-Phellandrene	1006	1.5	1.1
p-Cymene	1024	0.7	0.5
Limonene	1031	0.1	0.2
Camphene	1034	_	-
1,8-Cineole	1035	-	0.1
Phenylacetaldehyde	1044	0.3	0.4
Acetophenone	1067	1.5	-
Terpinolene	1085	-	0.1
Linalool	1101	1.2	0.5
Nonanal	1105	1.6	0.9
α-Terpineol	1180	0.1	-
2-Decanone	1190	0.1	-
Decanal	1203	1.8	2.1
Thymol methyl oxide	1230	0.3	-
Thymol	1290	-	1.2
Undecanal	1310	0.2	0.3
α-Cubebene	1350	-	0.1
α-Copaene	1375	2.3	3.1
β-Bourbonene	1385	0.1	-
β-Cubebene	1390	1.8	2.5
β-Caryophyllene	1418	9.5	3.3
β-Farnesene	1452	-	0.9
α-Humulene	1455	1.3	1.5
γ-Muurolene	1475	-	0.3
Germacrene D	1480	28.3	10.2
β-Selinene	1488	0.9	-
Bicyclogermacrene	1496	-	5.2
β-Bisabolene	1512	1.3	2.4
Spathulenol	1575	5.4	2.3
Caryophyllene oxide	1580	10.5	25.2
α -Cadinol	1640	2.7	0.3
β-Eudesmol	1650	5.3	11.5
α-Bisabolol	1680	2.5	-
Pentadecanal	1710	-	2.2
Hexadecanal	1815	1.3	4.2
Nonadecane	1905	-	0.2
cis-Phytol	2110	2.7	3.1
Tricosane	2295	-	0.5
Pentacosane	2495	1.2	-
Hexacosane	2600	0.2	0.1
Total		89.0	89.6

*RRI: Relative Retention Index

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Constituents	-	7	3	4	5	6	7	8	6	10	11	12 1	13	14 1	15 1	16 1	17	18 1	19	20 2	21 2	22	23	24 25		26
β-Caryophyllene	8.1	9.8	11.2	12.1	0.7	0.9	6.0	1.0	1.3	5.4 1							3.9	,					2.6			3.3
Germacrene D	36.0	44.3	29.4	44.2	ı	ı	ī		,	3.3 2	22.7 4	45.1 4(40.2 4	43.0 4(40.2 2	21.7 2	21.1	-	1.7 22	22.0 0	0.2 (0.6		1.7 28	28.3 1	0.2
Bicyclogermacrene	4.2	7.9	4.1	5.5	ı	ı			,	ı										1.7	ı	ı	ı			5.2
Caryophyllene oxide 4.1	4.1	3.1	1.9	3.0	7.8	6.2	10.3		10.0	4.7										3.2	1			1.5 10		5.2
Hexadecanoic acid	ı	ı	ı	ı	7.4	6.5				ı	·	ı								ı	ί. Έ					ı
β-Eudesmol	'	'	1.9	ī	ı	ı		-		_										1.2	,					1.5
Spathulenol	ı	'	ī	ī	3.8	4.2		0.9	4.9	3.9	0.8	3.3	1.0		2.2	2.2	0.7	6.3 10	10.8	ī	-	0.1 (0.2		5.4	2.3
β-Selinene	ı	0.9	0.8	3.1	ı	0.3																				ı
Tricosane	0.3	ц	0.3	3.6	0.3	2.3	0.8		ī	,											13.7 2	2.0	5.4	2.9		0.5
α-Cadinol	1.8	ı	ı	1.6	ı	2.2					ı											ı	ı			0.3
β-Bisabolene	0.4	ī	4.3	1.4	ı	ı	ī				1.3										ı	ı	ī	-		2.4
α-Copaene	1.5	1.6	1.0	1.1	ı	1.8	0.3	0.1		3.2	0.8	0.7							ı		,	ı	ī			3.1
α-Humulene	1.3	2.3	1.4	1.4	ı	ı	0.9				1.7									1.7 (0.1	ı		0.3 1		1.5
oc-Phellandrene	tr	1.1	0.3	tr	ı	ı	ı	ı	ı	ı	ı	,									ı	ı	,			1.1
œ-Pinene	tr	0.3	tr	tr	ı	ı	ı	0.1	1.8	·	tr	0.7			tr	tr		0.3	ı	ı	ı	ı	ı	-		1.4
1,8-Cineole	tr	tr	•	'	ı	ı	ı	ı	ı	ı	ı	,			ı	·		0.1	ı			ı	,	,		0.1
Thymol	ı	tr	1	tr	ı	ı	0.2	ı	·	·	tr	ŀ										2.4				1.2
Decanal	tr	2.2	I	0.2	0.6	1.4	0.1	ı	0.5	ı	0.6	0.6	1.2	,	0.4	0.5	0.2	-	0.4	1.3 (0.4	1.7 1	1.8	2.1
β-Cubebene	0.4	0.5	0.3	0.2	ı	0.4	0.1	1.7	ı	ı	tr	0.6	tr	-	0.4	tr	0.7	ı	ı	ı	ı	ı	t	-	1.8	2.5

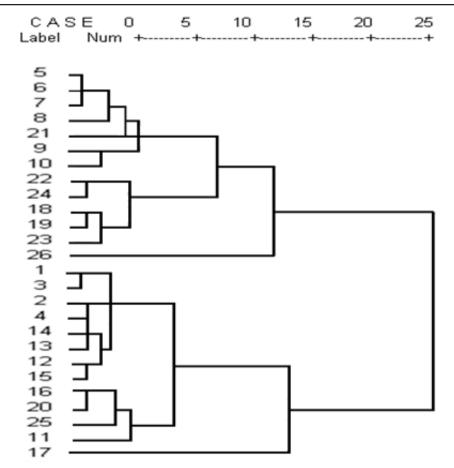
 C. pseudoscabiosa subsp. pseudoscabiosa and 2- C. hadimensis ²². 3- C. kotschyi var. decumbens and 4- C. kotschyi var. kotschyi ²³. 5- C. thessala subsp. drakiensis and 6- C. zuccariniana ²⁴. 7- C. raphanina subsp. mixta and 8- C. spruneri ²⁵. 9- C. sessilis and 10- C. armena ²⁶. 11- C. aladaghensis 12- C. antiochia var. prealta 13- C. antitauri 14- C. babylonica 15- C. balsamita 16-C. cheirolepidoides and 17- C. deflexa ²⁷. 18- C. cuneifolia and 19- C. euxina ²¹. 20- C. cineraria subsp. umbrosa and 21- C. napifolia ²⁸. 22- C. nicaeensis 23- C. parlatoris and 24- C. solstitialis L. subsp. schouwit ²⁹. 25- C. euxina ²¹. C. kurdica and 26- C. saligna (Studied samples)

var. kotschyi and C. kotschyi var. decumbens belongs to same section (Cheirolepis), also germacrene D (29.4 % - 44.2 % - 10.2 %) was the principal components of three taxa respectively. So we can say germacrene D is the chemotaxonomic marker for section Cheirolepis. It is noteworhy that C. napifolia essential oil showed different chemical properties from other investigated Centaurea taxa, includind high amounts of tricosane (13.7 %), small percentages of β caryophyllene (2.8 %) and germacrene D (0.2 %). Caryophyllene oxide and bicyclogermacrene weren't detected in this species. Furthermore tricosane was detected high amount (13.7%) only C. napifolia than other twenty five Centaurea taxa. C. antitauri, C. pseudoscabiosa subsp. pseudoscabiosa, C. hadimensis, C. kotschyi var. decumbens, C. kotschyi var. kotschyi, C. babylonica, C. antiochia var. prealta, and C. balsamita have more than 25 % of germacrene D, but their content of caryophyllene oxide was substantially smaller (2.8 %, 4.1 %, 3.1 %, 1.9 %, 3.0 %, 0.4 %, 0.8 %, and 0.4 % respectively). Whereas content of caryophyllene oxide in C. kurdica and C. saligna was significantly higher than above species (Table 2). C. cuneifolia, C. euxina, C. nicaeensis, C. parlatoris and C. solstitialis subsp. schouwii, C. thessala subsp. drakiensis, C. zuccariniana and C. raphanina subsp. mixta producing high amounts of hexadecanoic acid (17.6 %, 20.3 %, 33.5 %, 18.1 %, 29.4 %, 7.4 %, 6.5 % and 6.7 %, respectively) followed by small or no percentages of β -caryophyllene (-%, -%, 2.0%, 2.6%, 1.2%, 0.7%, 0.9 % and 6.0 %, respectively) and germacrene D (-%, 1.7%, 0.6%, -%, 1.7%, -%, -% and -%, respectively) (Table 2).

To appraise whether the reported essential oil compounds could be useful in reflecting the taxonomic relationships among the different *Centaurea* taxa, the components of all the essential oils were subjected to hierarchical cluster analysis (HCA). With this study, the chemotaxonomic importance and essential oil compounds in this genus was confirmed particularly with regard to the studied and referenced taxa. Results of cluster analysis (Figure 1) based on the distribution of essential oil show two main groups. One of them is a big group including (5, 6, 7, 8, 21, 9, 10, 22, 24, 18, 19, 23, 26) samples. The other group includes (1, 3, 2, 4, 14, 13, 12, 15, 16, 20, 25, 11, 17) samples. Furthermore we can separate first main group in three small groups. In fact, in the dendrogram among first main taxa, C. saligna was very far apart from all the other taxa. Also among second main taxa, C. deflexa was very far apart from all the other taxa. Chemical dendrogram obtained by cluster analysis of the percentage composition of essential oils from Centaurea taxa showed that C. thessala subsp. drakiensis, C. zuccariniana, C. raphanina subsp. mixta (Sect. Acrocentron), C.spruneri, C.napifolia, C.sessilis (Sect. Rhizocalathium), C. armena (Sect. Rhizocalathium), samples were closest to C. nicaeensis, C. solstitialis subsp. schouwii (Sect. Mesocentron), C. cuneifolia (Sect. Acrolophus), C. euxina (Sect. Phalolepis), C. parlatoris samples and they were related with the C. saligna (Sect. Cheirolepis) and C. pseudoscabiosa subsp. pseudoscabiosa (Sect. Acrocentron), C. kotschyi var. decumbens (Sect. Cheirolepis), C. hadimensis, C. kotschyi var. kotschyi (Sect. Cheirolepis), C. babylonica (Sect. Microlophus), C. antitauri (Sect. Pseudophaeopappus), C. antiochia var. prealta, C. balsamita (Sect. Stizolophus) species were closest to C. cheirolepidoides (Sect. Pseudoseridia), C. cineraria subsp. umbrosa, C. kurdica (Sect. Cynaroides), C. aladaghensis (Sect. Cynaroides) and they were related with the C. deflexa (Sect. Cheirolepis).

In conclusion, *C. saligna* and *C. kurdica* synthesized many same constituents in their essential oils that could be justified by the similar ecological and habitat conditions. The comparison between two taxa evidenced a similarity, at least with reference to the presence of the main constituents: in fact germacrene D and caryophyllene oxide was among the principal one in both taxa. Also the percentages of caryophyllene, spathulenol and β -eudesmol were comparable. The only differences between two species were substantially due to bicyclogermacrene: found only in *C. saligna* (5.2 %) (Table 1).

This study demonstrates the occurrence of Germacrene D / caryophyllene oxide chemotype of *C. kurdica* and caryophyllene oxide / β -



1-C. pseudoscabiosa subsp. pseudoscabiosa and 2-C. hadimensis ²². 3-C. kotschyi var. decumbens and 4-C. kotschyi var. kotschyi ²³. 5-C. thessala subsp. drakiensis and 6-C. zuccariniana ²⁴. 7-C. raphanina subsp. mixta and 8-C. spruneri ²⁵. 9-C. sessilis and 10-C. armena ²⁶. 11-C. aladaghensis 12-C. antiochia var. prealta 13-C. antitauri 14-C. babylonica 15- C. balsamita 16-C. cheirolepidoides and 17- C. deflexa ²⁷. 18- C. cuneifolia and 19-C. euxina ²¹. 20-C. cineraria subsp. umbrosa and 21-C. napifolia ²⁸. 22-C. nicaeensis 23-C. parlatoris and 24-C. solstitialis L. subsp. schouwii ²⁹. 25-C. kurdica and 26-C. saligna (Studied samples)

Figure 1. Hierarchical cluster analysis of twenty six Centaurea taxa

eudesmol chemotype of *C. saligna* in Eastern Anatolian region of Turkey. Some of the *Centaurea* species showed different chemotype of essential oil, like Germacrene D chemotype in *C. mucronifera* and *C. chrysantha*³⁰, β -eudesmol chemotype in *C. sessilis* and *C. armena*²⁶, β -eudesmol / hexadecanoic acid chemotype in *C. cuneifolia* and hexadecanoic acid / spathulenol chemotype in *C. euxina*²¹. The essential oil results have given some clues on the chemotaxonomy of the genus patterns and usability of the oils as natural product and oil resource plant.

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