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Essential Oil Composition of Endemic *Cyclotrichium niveum* (Boiss.) Manden & Scheng (Lamiaceae) from Turkey: A Chemotoxonomic Approach

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In this study, hydrodistilled essential oil derived from the aerial parts of *Cyclotrichium niveum* (Boiss.) Manden. & Scheng (Lamiaceae) grown in Turkey were analyzed by GC and GC-MS system. Fourty seven components were identified representing 94.2 % of the oil. It was determined that *C. niveum* essential oil contained pulegone (32.0 %), germacrene-D (16.9 %), menthone (16 %), isomenthone (8.8 %) and spathulenol (3.4 %) as major compounds, respectively. Essential oil analysis of the *Cyclotrichium niveum* has shown that it has pulegone/germacrene-D and menthone chemotype. The results were discussed by using numerical taxonomic clustering techniques in means of chemotaxonomy with the genus patterns. The dendrogram showed that the essential oil composition of *Cyclotrichium* genus patterns have different content but similar profiles in view of major compounds.

Keywords: *Cyclotrichium niveum*, Pulegone, Germacrene-D, Essential oil, Chemotaxonomy.

INTRODUCTION

The genus *Cyclotrichium* (Boiss.) Manden. & Scheng is represented by six species in Turkey: *C. niveum*, *C. origanifolium*, *C. leucotrichum*, *C. stamineum*, *C. glabrescens* and *C. longiflorum*. They are aromatic perennial subshrubs. Two of them with this species are endemic to Turkey (*C. niveum* and *C. glabrescens*) [1,2]. It is represented in Lamiaceae family which includes 45 genera and 546 species and totally 731 taxa in the Flora of Turkey. Endemism of Lamiaceae is 44.2 %, which is the third richest family in Turkey [1]. Lamiaceae family is one of the few plants families includes numerous genera and species used as culinary vegatative, herbal medicine and also as great ornamental interest, extensively in planning of parks, gardens and urban green areas [3]. The flora of Turkey is estimated to contain over 3000 aromatic plants. Among to aromatic taxa, remarkable examples can be found in families such as Labiatae, Compositae, Apiaceae, *etc*. Aromatic diversity is illustrated with examples from genera such as *Sideritis, Salvia, Thymus, Origanum, Satureja, Thymbra, Mentha, Teucrium, Ziziphora, Calamintha* and *Cyclotrichium* in Lamiaceae and the essential oils of Lamiaceae members growing in Turkey have been more published [4-6].

All *Cyclotrichium* species are Irano-Turanian elements except for *Cyclotrichium origanifolium* [7]. *C. origanifolium* is variable in habit, indumentum and flower size, being apparently differentiated into several local races. *C. leucotrichum* is related to *C. stamineum*. *C. stamineum* is very closely related to both *C. glabrescens* and *C. longiflorum*. *C. glabrescens* is very close to *C. stamineum* [1]. Some members of genus *Cyclotrichium* are used to make herbal teas and as flavouring agents in soups and salads in Turkey. *Cyclotrichium niveum* known as "dag nanesi" in Turkish, is an endemic species growing in the eastern Anatolia [8-10]. *C. niveum* (syn. *Calamintha nivea* Boiss.) has rich essential oil content, dominated usually by pulegone and is well-known that most spices possess a wide range of biological and pharmacological activities [10]. Goze *et al.* [11], were designed to examine *in vitro* antimicrobial and antifungal activities of the essential oil of *C. niveum.* To the best of our knowledge, there is limited number of reports on the genus *Cyclotrichium* [9,12-14].

The morphology of glandular and non-glandular trichomes and the essential oil composition of Lamiaceae play an important role in the ecology of these species as well as for their industrial use. They may also serve as taxonomic criteria [15]. Morphological, karyological and phylogenetic evaluation of *Cyclotrichium* genus showed that the combined results strongly suggested that it is a seperate genus in Nepetoideae with distinct morphological, phylogenetic and cytogenetic characteristics. For intrageneric phylogeny of *Cyclotrichium*, three groups were recognized: (1) *C. niveum* (2) *C. origanifolium* and (3) the remaining six species [16]. Some phytochemicals have chemotaxonomic importance in the plant families. The diterpenes had been used as chemotaxonomic markers at infra- and supra-genus levels in some Lamiaceae study [17], flavonoids [18] essential oils [19] and fatty acids are used for chemotaxonomic evaluation of the Lamiaceae genera patterns [20]. This study was carried out to determine the essentail oil composition of *C. niveum* from eastern Anatolian region, Turkey and to discuss the chemical variation among the genus patterns of *Cyclotrichium* in view of chemotaxonomy.

EXPERIMENTAL

Cyclotrichium niveum were collected from Kemaliye, Erzincan, 2100 m in 2008. Voucher specimens are kept at the Firat University Herbarium (FUH).

Isolation of essential oils: Air-dried aerial parts of the plant material (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h to yield.

Gas chromatographic analysis: The essential oil was analyzed using HP 6890 GC equipped with and FID detector and an HP-5 MS column (30 m × 0.25 mm *i.d.,* film tickness 0.25 µm) capillary column was used. The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

Gas chromatography/mass spectrometry (GC-MS) analysis: The oils were analyzed by GC-MS, using a Hewlett Packard system. HP-Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Res. Lab. (BUBAL) in Firat University. HP-5 MS column (30 m × 0.25 mm *i.d.,* film tickness 0.25 µm) was used with helium as the carrier gas. Injector temperature was 250 °C, split flow was 1 mL/min. The GC oven temperature was kept at 70 °C for 2 min. and programmed to 150 °C at a rate of 10 °C/min and then kept constant 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. Component identification was carried out using spectrometric electronic libraries (WILEY, NIST). The identified constituents of the essential oils are listed in Table-1.

The essential oil compounds of the *C. niveum* studied and the reports from different studies [21,22] on the various *Cyclotrichium* species were evaluated by means of Numerical taxonomical techniques using Cluster analysis in SPSS 15.0 packet program. The dendrogram (Fig. 1) of the essential oil compounds in *Cyclotrichium* genus patterns were obtained by using major compounds determined and reported in different *Cyclotrichium* species (Table-2).

TABLE-1
CONSTITUENTS OF THE ESSENTIAL OIL FROM *Cyclotrichium niveum*

No.	Compounds	RRI	*C. niveum*
1	Heptane	897	1.0
2	Toluen	910	0.1
3	*n*-Hexanal	917	0.1
4	Bicyclo[3.1.0]hex-2-ene	969	0.1
5	α-Pinene	974	0.4
6	Camphene	984	0.1
7	β-Phellandrene	998	0.2
8	β-Pinene	1002	0.5
9	β-Myrcene	1008	0.2
10	Limonene	1040	1.0
11	Eucalyptol	1043	2.4
12	*cis*-β-Terpineol	1074	0.1
13	Bicyclo[4.1.0]hept-2-ene	1086	0.1
14	*trans*-Linalooloxide	1087	0.1
15	Linalool	1100	0.1
16	β-Thujone	1116	0.1
17	Limonene oxide	1133	0.1
18	Cyclohexanone	1152	0.6
19	Menthone	1167	**16**
20	Isopulegone	1173	2.1
21	3-Cyclohexen-1-ol	1177	0.1
22	Pulegone	1244	**32**
23	Isomenthone	1246	**8.8**
24	Bicyclo[2.2.1]heptan-2-ol	1278	0.1
25	Thymol	1287	0.7
26	2-Cyclohexen-1-ol	1325	0.1
27	2,4-Cycloheptadien-1-one	1330	0.7
28	Copaene	1366	0.1
29	Cyclobuta[1,2;3,4]dicyclopentene	1374	0.1
30	2-Cyclopenten-1-one	1383	0.1
31	β-Caryophyllene	1408	0.1
32	(+)Epibicyclosesquiphellandrene	1418	0.1
33	2-Cyclohexen-1-one	1428	**3.1**
34	1H-Cycloprop[e]azulene	1446	0.1
35	Germacrene-D	1465	**16.9**
36	Bicyclogermacrene	1481	0.3
37	Sphathulenol	1557	**3.4**
38	Valerenol	1562	0.1
39	Isospathulenol	1604	0.6
40	Neoisolongifolene	1616	0.1
41	2-Naphthaleneethanol	1791	0.4
42	Salvial-4[14]-en-1-one	1831	0.1
43	*n*-Hexadecanoic acid	1870	0.1
44	Pentacosane	1979	0.1
45	3-Octadecanoic acid	1996	0.1
46	Heptacosane	2056	0.1
47	Cholesterol	2131	0.3
	Total		**94.2**

RRI: Relative retention index

RESULTS AND DISCUSSION

The essential oils of the aerial parts of *Cyclotrichium niveum* collected from the eastern Anatolian region of Turkey were obtained by hydrodistillation, in 0.9 % (v/w) yield. The result of essential oils analysis are presented in Table-1. Overall, fourty seven compounds which accounted for 94.2 % in *Cyclotrichium niveum*. The oils were complex mixtures of monoterpenes and sesquiterpenes. The major compounds of essential oil studied were pulegone (32 %), germacrene-D (16.9 %), menthone (16.0 %), isomenthone (8.8 %) and spathulenol (3.4 %) respectively. 2-Cyclohexen-1-one, eucalyptol, isopulegone, limonene and heptane were determined as minor compounds in the essential oil of *C. niveum* studied (Table-1). The major constituents of the essential oil of *C. niveum* reported as pulegone (50.46 %) and iso-menthone (34.53 %) in Goze *et al.* [11] study. There were also many other compounds in minor amounts.

The oil obtained from the aerial parts of *Cyclotrichium niveum* is determined to contain a high percentage of pulegone

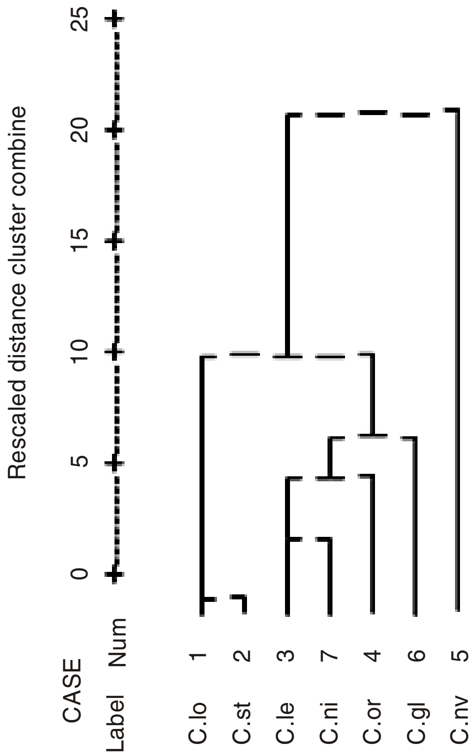

Fig. 1. Dendogram of *Cyclotrichium* species

(32 %). This constituent is also reported as high amounts in *C. depauperatum* (9.8 %) [23], *C. leucotrichum* (15.6 %) and *C. origanifolium* (14.1 %) [21], respectively. The percentage of the spathulenol was found as 3.4 % in *C. niveum* essential oil studied here (Table-1). Some species of genus *Cyclotrichium* were rich in spathulenol content like in *C. longiflorum* (2.81, 6.8 %) [21,24], *C. glabrescens* (6.1 %) and *C. leucotrichum* (3.1 %) [21]. Menthone (16.0 %) is also one of the major component in *C. niveum* essential oil (Table-1) and it was reported in *C. origanifolium* as 25.2 % and 32.5 % [21,25] in *C. depauperatum* as 4.5 % [23], in *C. stamineum* as 4.3 % and in *C. longiflorum* as 3.6 % [21]. Isomenthone (8.8 %) is another major component determined in this study (Table-1) and it is reported as 6.65 % in *C. niveum* essential oil from Sivas, Turkey [22]. Germacrene-D (16.9 %) was detected as one of the major components in the essential oil of *C. niveum* (Table-1), however it was not found in *C. longiflorum*, *C. stamineum*, *C. leucotrichum*, *C. origanifolium*, *C. glabrescens* [21] and *C. niveum* [22]. In another study on the essential oil composition of *Cyclotrichium niveum* from Sivas, Gürün, was reported previously and the main components of the species were reported pulegone (32.49-56.56 %) and isomenthone (33.75-35.36 %). The main compounds were determinend as pulegone in this study. However, the percantage of pulegone in the essential oil was 81.2 % and isomenthone was found as a trace. This difference could depend on the climate conditions of the collection years [10]. Our analysis results study were showed similarity with the Baser's findings, because the pulegone and isomenthone were major components in our samples. According to GC/MS results of the essential oil *Cyclotrichium niveum* by Cetinus *et al*. [22], thirty two compounds, representing 94.82 % of the oil were identified, with pulegone (76.84 %), isomenthone (6.65 %) and isopulegone (3.01 %) being the major constituents [22]. Our analysis results study were showed similarity with this study, because of the pulegone and isomenthone majority in our samples.

Thymol, carvacrol, isopulegol acetate, menthol acetate were reported as the major components of essential oil of *C. glabrescens* [21] but they were not among the major compounds of other *Cyclotrichum* species. β-Pinene is the major component of *C. origanifolium* [21] and *C. longiflorum* [24], except *C. niveum*, *C. stamineum*, *C. leucotrichum* and *C. glabrescens* [21]. However 1,8-cineole and β-caryophyllene are the major components of essential oil of *C. origanifolium* and *C. stamineum*, respectively [21].

It is reported that the aerial parts of *C. depauperatum* has yielded 0.3 % (v/w) of a yellowish oil with an aromatic odor. Forty six components were detected in this plant essential oil. The major components of the oil are *cis*-pinocamphone (19.1 %), pulegone (9.8 %), linalool (9.4 %), *cis*-isopulegone (9.3 %), linalyl acetate (8.8 %), menthone (4.5 %) and α-terpinyl acetate (3.4 %). The oil of *C. depauperatum* consisted of eleven

TABLE-2
MAJOR COMPOUNDS OF ESSENTIAL OIL OF *Cyclotrichium* SPECIES USED IN THE CLUSTER ANALYSIS [Ref. 21,22]

Major compounds (%)	*C. glabrescens*	*C. longiflorum*	*C. stamineum*	*C. leucotrichium*	*C. origanifolium*	*C. niveum*
Thymol	26.3	Tr.	0.3	Tr.	Tr.	Tr.
Carvacrol	16.6	Tr.	Tr.	Tr.	Tr.	Tr.
Isopulegol acetate	11.6	Tr.	Tr.	Tr.	Tr.	Tr.
Menthol acetate	7.5	-	-	-	-	-
Spathulenol	6.1	6.8	6.0	3.1	0.7	1.1
Isopinacamphone	Tr.	59.8	47.4	1.7	26.4	0.3
Myrtenyl acetate	Tr.	6.7	3.9	Tr.	0.8	0.1
p-Mentha-3,8-diene	Tr.	-	-	15.8	Tr.	Tr.
t-Cadinol	-	1.4	1.3	15.8	Tr.	0.1
Pulegone	1.2	Tr.	Tr.	15.6	14.1	81.2
Borneol formate	-	-	-	7.6	Tr.	0.3
Terpinen-4-ol	1.2	1.1	7.0	5.9	Tr.	0.7
Menthone	0.8	3.6	4.3	Tr.	25.2	Tr.
β-Pinene	1.2	Tr.	1.1	Tr.	15.5	0.2
1,8-Cineole	Tr.	0.5	0.5	2.7	8.9	0.9
Aromadendrene	1.2	1.7	3.9	Tr.	Tr.	Tr.
β-Caryophyllene	0.7	0.8	4.8	2.4	Tr.	Tr.
Linalool	Tr.	1.0	1.5	Tr.	0.3	0.1

monoterpene hydrocarbons (5.8 %), 20 oxygenated monoterpenes (80.6 %), seven sesquiterpene hydrocabons (7.1 %) and seven oxygenated sesquiterpenes (3.2 %) [23]. The oil of *C. niveum* studied here also contains monoterpenes and usually found abundantly in *Cyclotrichium* genus pattern oils. In the hydrodistilled essential oil of *C. glabrescens* was analyzed and 29 volatile compounds were identified which represented 75.6 % of the total oil. The main compunds of the essential oil were found to be thymol (26.3 %), carvacrol (16.6 %), isopulegol acetate (11.1 %) and spathulenol (6.1 %). On the other hand, thirty-one components were determined in the essential oil of *C. longiflorum* which represented 90.2 % of total oil and isopinocamphone (59.8 %), spathulenol (6.8 %), myrtenyl acetate (6.7 %) and menthone (3.6 %) were the main compounds [21]. In the essential oil of C. *longiflorum* Leblebici sixteen components were found comprising 98.04 % of the total oil. Isopinocamphone (67.66 %), β-pinene (9.67 %), limonene (4.30 %) and spathulenol (2.81 %) being the major constituents reported [24,25].

The essential oil composition of *Cyclotrichium leucotrichum,* consists of 36 compounds representing 82.3 % of the total oil. The main compounds were distributed in equal percentages in contrast to other species were the compounds are present in different percentages; *p*-mentha-3,8-diene and *t*-cadinol (15.8 %), pulegone (15.6 %), borneol formate (7.6 %), terpinen-4-ol (5.9 %), spathulenol (3.1 %) and *trans*-caryophyllene (2.4 %) [21]. However, according to Baser *et al*. [10], the main compounds of the species obtained from the Herbarium of the Royal Botanic Garden, Edinburgh were reported again to be present in different percentages; caryophyllene (14.4 %), *p*-menth-3-en-8-ol (11.1 %) and camphor (11.92 %). In the essential oil analysis of *Cyclotrichium origanifolium* collected from different region of Turkey; the results are similar to the other species collected from different localities. The main compounds were determinend as isopinocamphone (26.4 %), menthone (25.2 %), β-pinene (15.5 %), pulegone (14.1 %) and 1.8-cineole (8.9 %) [21].

The dendrogram (Fig. 1) based on the essential oils major compounds of *Cyclotrichium* species (studied in here-7 numbered) and reported from different studies on the *Cyclotrichium* species essential oil (Table-2) results showed that three groups are in the clustering of this genus. *C. longiflorum* and *C. stamineum* were found as similar essential oil as placed in the same group (1,2) according to the major compounds. The similarity between two species is also reported by Davis [1] in view of morphology. The other big group were comprised of *C. leucotrichium, C. niveum* (this study results-7), *C. origanifolium, C. glabrescens* species. The dendrogram showed that *C. longiflorum* and *C. stamineum* were very similar and the other 4 species mentioned above were similar each other but not more than to the both species and *C. niveum* reported by Kilic *et al*. [21] (the last alone cluster). *C. niveum* is reported as very distinct from other *Cyclotrichium* species on account of its white, dendroid indumentum [1], we also determined that it has more infra-generic and spesific chemical variation in the genus. The dendrogram and Kilic *et al*. [21], study on the *C. niveum* showed that the essential oils of genus patterns has more variation infrasepesific means. In the study of Satil *et al*. [26] on the taxonomic value of leaf anatomy and trichome morphology of the genus *Cyclotrichium* in Turkey, revealed that peltate trichomes are densely spaced only on the calyx and on the leaf surface of *C. niveum* and *C. origanifolium* and on the abaxial leaf surface of *C. longiflorum* and *C. stamineum* [26]. This report on the taxonomy of genus patterns supported the dendrogram results, particularly in view of chemical similarity.

The results of the essential oil analysis of *C. niveum* from the eastern Anatolian region has revealed that it has pulegone/germacrene-D and menthone type essential oil. It can be also said that, *C. longiflorum* has isopinochamphone/spathulenol and myrtenyl acetate type; *C. stamineum* has isopinocamphone/terpinen-4-ol and spathulenol type; *C. origanifolium* has isopinochamphone/menthone and β-pinene type; *C. leucotrichium* has *p*-mentha-3,8-diene/*t*-cadinol and pulegone type; *C. glabrescens* has thymol/carvacrol and isopulegol acetate type essential oils according to the literature reviews of *Cyclotrichium* species (Table-2).

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