ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. X:X (20XX) XX-XX

records of natural products

Composition of the Essential Oils of Two Endemic *Helichrysum* Species in Turkey

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(Received Received June 10, 2018, Revised July 30, 2018; Accepted August 09 2018)

Abstract: The aerial parts of *Helichrysum noeanum* Boiss. and *H. chionophilum* Boiss. et Balansa were hydrodistilled for 3 h using a Clevenger-type apparatus. The essential oils were analyzed by GC-FID and GC-MS, simultaneously. The main constituents were identified as hexadecanoic acid (25.3%), dodecanoic acid (17.8%), tetradecanoic acid (13.7%), and decanoic acid (4.2%) for *H. noeanum*. Decanoic acid (18.7%), tetradecanoic acid (16.9%), dodecanoic acid (13.8%), hexadecanoic acid (12.2%) and hexahydrofarnesyl acetone (3.2%) were found to be the major compounds for *H. chionophilum*. Several reports have been encountered in the literature dealing with the oil composition of several *Helichrysum* species. To the best of our knowledge, the essential oil of *H. chionophilum* has not previously been investigated.

Keywords: *Helichrysum noeanum*; *H. chionophilum*; essential oil; GC; GC-MS.

1. Introduction

The genus *Helichrysum* Mill. belongs to the Asteraceae consists of approximately 600 species widespread throughout the world. Its name was derived from the Greek words *helios* (sun) and *chrysos* (gold) which is appropriate considering the attractive yellow flowers displayed by several species [1]. Most species are indigenous to South Africa (approximately 250 species), some are spontaneous in Europe (16 species) and in the Mediterranean areas. Some species are found in South-west Asia, southern India, Sri Lanka and Australia [2]. In Turkey, this genus is represented by 30 taxa. Sixteen of them are endemic in Turkey and *Helichrysum* species are known as "altın çiçeği," altınotu," and "kovanotu" [3].

Members of the genus *Helichrysum* are aromatic herbs or dwarf perennial shrubs [2]. The species have been used in folk medicine for at least 2000 years against gall bladder disorders [4]. The

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The article was published by ACG Publications
www.acgpubs.org/RNP © Month-Month 2018 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.93.18.06.113

species are used in the folk medicines of Europe and Africa as herbal teas for the treatment of cough and respiratory problems, digestive disorders, skin inflammation, fever, wound care and pain with analgesic effect. Furthermore, the species are often used to treat tuberculosis and for applications as cardiotonic, diuretic and anti-diarrheal remedies [2,5]. In Turkey, several *Helichrysum* species are utilized in folk medicine for removing the kidney stones and as diuretics [4]. Herbal teas are consumed for various biological properties including anti-inflammatory, antioxidant and antimicrobial activity in Turkey and various parts of the world [6].

The genus has an important source of secondary metabolites such as flavonoids, phytocannabinoids, triterpenoids, diterpenoids, steroids, organic acids, phloroglucinol and acetophenone derivatives and aromatic species have been studied for their content of essential oils [6-10]. Several reports have been encountered in the literature dealing with the oil composition of several *Helichrysum* species; these include *H. bracteiferum* (DC.) Humbert [11], *H. picardii* Boiss. & Reuter [12,13], *H. taenari* Rothm. and *H. stoechas* (L.) Moench ssp. *barrelieri* (Teno) Nyman [13] and *H. amorginum* Boiss. & Orph. and *H. italicum* (Roth) G. Don [14], *H. cordifolium* DC., *H. hypnoides* (DC.) R.Vig. et Humbert, *H. rusillonii* Hochr. [15], *H. dasyanthum* (Willd.) Sweet, *H. excisum* (Thunb.) Less and *H. felinum* Less and *H. petiolare* Hilliard and Burtt [16], *H. forsskahlii* (Gmel) Hilliard et Burt [17], *H. plicatum* DC. (*H. plicatum* DC. subsp. *plicatum*, *H. plicatum* DC. subsp. *polyphyllum* (Ledeb) P.H.Davis & Kupicha and *H. plicatum* DC. subsp. *isauricum* Parolly) [18], *H. conglobatum* (Viv.) Steudel. [19], *H. noeanum* Boiss. [20].

Helichrysum species have been known since ancient times due to a large distribution and diversity [21]. *Helichrysum* is a rather variable genus, since a number of modifications strongly affect the morphological aspect of its species and consequently the taxonomic determination is often uncertain [22]. Along with the taxonomically recognized *Helichrysum* species a significant number of evolved hybrids are also found. The individual morpho-anatomical characteristics of these hybrids can be deceiving and can lead to wrong assignments to recognized taxa. The high degree of genotypic variability, observed in a number of *Helichrysum* species, is rejected in the biochemical variability which is usually studied at the levels of terpene composition and isozyme variation. The volatile constituents, especially monoterpenes, can be used for taxonomic purposes [23].

Among the large number of phytoproducts, isolated from *Helichrysum* species, essential oil has an important biological role. Since, the plant is characterized by a high level of genetic and metabolic polymorphism forming as a response to environmental conditions and geographic location, different chemotypes of its essential oils can be expected [24].

In the literature, there are a few published data on *H. noeanum* and *H. chionophilum*. The essential oil composition of the aerial parts [25] and flavonoids of capitula and leafy stems [26] of *H. noeanum* were reported. 3,5-Dihydroxy-6,7,8-trimethoxyflavone, apigenin, kaempferol, naringenin, helichrysin A, helichrysin B, isosalipurposide, naringenin 4'-glucoside, apigenin 7-glucoside, and astragalin were identified in the capitula whereas kaempferol, astragalin, and quercetin 3-glucoside were isolated from the leafy stems [26]. Elkıran et al. [25] reported the major compounds as γ-gurjunene (11.06%), spathulenol (9.90%), alloaromadendrene (7.53%), β-caryophyllene (7.10%) in the essential oil of aerial parts. No study has been encountered on phytochemistry of *H. chionophylum*.

These endemic species were evaluated for their possible biological activities by limited studies. Tepe et al. [27] evaluated *in vitro* antioxidant activities of *Helichrysum noeanum* and *H. chionophilum* methanol extracts. Eroglu et al. [28] found the genotoxic activities of *H. noeanum* methanol extracts by inducing the formation of micronuclei and decreasing the mitotic and replication indexes. It was published by Albayrak et al. [4] that *H. noeanum* extract showed significant antimicrobial activity.

In the present study, endemic species *Helichrysum noeanum* and *H. chionophilum* were studied for their volatile compound characterizations. *H. noeanum* and *H. chionophilum* are locally known as “gulazar and yaylahencecaligi”, resp. [29]. The essential oil composition of *H. chionophilum* was characterized for the first time.

2. Materials and Methods

2.1. Plant Material

Aerial parts of *Helichrysum noeanum* (A) and *H. chionophilum* (B) were collected while flowering from Ankara: Bala-Kaman on June 30, 2005 and Kayseri: Arslantaş plateau on August 14, 2007, resp. Voucher specimens were kept at the herbarium of the Faculty of Science (Aksoy 2100 and Aksoy 2089) resp., Akdeniz University (Turkey).

2.2. Isolation of the Essential Oils

The dried aerial parts of *Helichrysum noeanum* and *H. chionophilum* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus.

2.3. GC and GC-MS Conditions

The oils were analyzed by capillary Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) using a Agilent GC-MSD system (Mass Selective Detector-MSD) (Agilent Technologies Inc., Santa Clara, CA).

2.3.1. GC-MS Analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system according to the literature [15]. Innowax FSC column (60m x 0.25mm, 0.25µm film thickness) was used with helium as carrier gas (0.8 mL/min.). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted 40:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450.

2.3.2. GC Analysis

The GC analysis were done according to the literature [15] was carried out using an Agilent 6890N GC system. In order to obtain same elution order with GC-MS, simultaneous injection was done by using same column and an appropriate operational conditions. FID temperature was 300°C.

2.4. Identification of compounds

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Adams Library [30], MassFinder Library [31], Wiley GC-MS Library [32] and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Alkanes were used as reference points in the calculation of relative retention indices (RRI) [33]. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analysis are shown in Table 1.

3. Results and Discussion

The present work reports the composition of the essential oils of *Helichrysum noeanum* and *H. chionophilum*, endemic in Turkey. The aerial parts of *H. noeanum* and *H. chionophilum* were water distilled for 3 h using a Clevenger-type apparatus. The yields of *H. noeanum* and *H. chionophilum* essential oils were 0.04% and 0.07%, resp. The essential oils were analyzed by GC-FID and GC-MS simultaneously. Forty one and forty-seven volatile compounds were identified in both of the aerial parts essential oils representing 83.2% and 84.6% of the total oils, respectively.

Table 1. The composition of the essential oils of *Helichrysum noeanum* and *H. chionophilum*

RRI[a]	RRI[b]	Compounds	A %	B %	IM
1400	1400[k,m]	Tetradecane	-	0.1	t_R
1401	1400[c], 1391[d]	Nonanal	-	0.2	MS
1497	1497[c], 1488[e]	α-Copaene	0.6	0.1	MS
1500	1500[m]	Pentadecane	-	0.1	t_R, MS
1506	1506[c], 1496[d]	Decanal	-	0.3	MS
1553	1543[d]	Italicene	-	0.3	t_R, MS
1562	1562[k, n]	Octanol	-	0.1	t_R, MS
1612	1612[c], 1598[d]	β-Caryophyllene	0.5	1.2	t_R, MS
1628	1620[d]	Aromadendrene	-	0.2	MS
1661	1664[d]	α-Himachalene	-	0.2	MS
1664	1656[d]	Nonanol	-	0.3	MS
1687	1663[e],1667[d]	α-Humulene	0.7	0.2	t_R, MS
1704	1704[f,k], 1689[g]	γ-Muurolene	0.6	-	MS
1711	1709[d]	γ-Himachalene	-	0.2	MS
1719	1719[k]	Borneol	tr	0.2	t_R, MS
1722	1712[d]	Dodecanal	-	0.1	t_R, MS
1729	1723[d]	β-Himachalene	-	0.1	MS
1740	1740[f], 1723[g]	α-Muurolene	0.4	tr	MS
1744	1725[d]	α-Selinene	0.1	-	MS
1765	1748d	*(E)*-2-Undecenal	-	0.4	MS
1772	1773[f,k], 1755[g]	δ-Cadinene	0.7	0.1	t_R, MS
1776	1776[f,k], 1763[g]	γ-Cadinene	0.6	0.1	MS
1786	1774[d]	*ar*-Curcumene	-	0.1	MS
1811	1769[d]	α-Cadinene	0.2	-	MS
1853	1835[d], 1849[g,k]	*cis*-Calamenene	0.3	-	MS
1868	1855[d]	(*E*)-Geranyl acetone	0.2	0.6	t_R, MS
1882	1884[p]	1-Isobutyl 4-isopropyl-2,2-dimethyl succinate	0.2	0.6	MS
1941	1921[d,g], 1941[f]	α-Calacorene	0.2	-	MS
1965	1866[d], 1972[p]	Dodecanol	0.2	-	MS
2008	1986[d], 2008[c,p]	Caryophyllene oxide	1.4	0.3	t_R, MS
2041	2015[d], 2041[d]	Pentadecanal	-	0.4	MS
2045	2045[g]	Humulene epoxide-I	0.4	-	MS
2071	2047[d,g], 2069[f]	Humulene epoxide-II	1.5	0.2	MS
2073	2044[d]	β-Caryophyllene alcohol	0.4	0.5	MS
2080	2074[d]	1, 10-diepi- Cubenol	tr	-	MS
2081	2081[g]	Humulene epoxide-III	0.1	-	MS
2084	2057[d]	Octanoic acid	-	0.2	MS
2131	2131[c], 2124[e]	Hexahydrofarnesyl acetone	1.7	4.9	t_R, MS
2162	2146[r]	Muurola-4,10(14) dien-1-ol	0.3	-	MS
2174	2159[d]	Nonanoic acid	0.4	0.8	MS
2191	2187[f], 2165[g]	T-Cadinol	0.6	0.4	MS
2204	2205[c], 2164[d]	Thymol	-	0.1	t_R, MS
2205	2196[p]	Clovenol	0.2	0.9	t_R, MS
2209	2209[f,p], 2140[h]	T-Muurolol	0.2	-	MS
2219	2219[r]	δ-Cadinol	0.2	-	MS
2246	2246[c], 2211[d]	Carvacrol	0.5	2.1	t_R, MS
2255	2255[f], 2227[d,g]	α-Cadinol	0.8	-	t_R, MS
2256	2233[d]	Cadalene	-	0.2	MS
2286	2274[d]	**Decanoic acid**	4.2	**18.9**	MS
2300	2300[m]	Tricosane	0.7	-	t_R, MS
2324	2324[g]	Caryophylladienol II	-	0.3	MS
2353	2389[g]	Caryophyllenol I	-	0.8	MS
2392	2392[c,d]	Caryophyllenol II	-	0.8	MS
2396	2396[p]	γ-Dodecalactone	0.3	0.5	MS
2399	2391[d]	Undecanoic acid	1.1	0.3	t_R, MS

Table 1 Continued..

2400	2400[m]	Tetracosane	0.6	-	t_R, MS
2500	2500[m]	Pentacosane	3.8	-	MS
2503	2487[d], 2503[g]	**Dodecanoic acid**	**17.8**	**13.9**	t_R, MS
2617	2617[d]	Tridecanoic acid	-	0.7	MS
2696	2696[c], 2687[d]	**Tetradecanoic acid**	**13.7**	**17.0**	t_R, MS
2819	2822[d]	Pentadecanoic acid	1.0	1.6	MS
2859	2765[p]	γ-Palmitolactone	0.5	0.7	MS
2931	2931[c,g], 2913[d]	**Hexadecanoic acid**	**25.3**	**12.3**	MS

RRI[a]; Relative retention indices calculated against n-alkanes. %; calculated from the FID chromatograms. RRI[b]: : RRI from literature (c [34], d [35], e [36], f [37], g [38], h [39], k [40], m [41], n [42], p [43], r [44]) for polar column values; tr; Trace (<0.1 %). Identification method (IM): t_R, identification based on the retention times (t_R) of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data. A: *Helichrysum noeanum* Boiss. B: and *H. chionophilum* Boiss. et Balansa

Aerial parts of Madagascarian *H. bracteiferum* (DC.) Humbert: 1,8-cineole (24.8%) and α-humulene (10.1%), *H. cordifolium* DC.: β-caryophyllene (46.4%) and α-humulene (10.9%), *H. hypnoides* (DC.) R.Vig. et Humbert: 1,8-cineole (51.5%) and α-terpineol (13.2%), *H. rusillonii* Hochr.: β-caryophyllene (29.5%) and 1,8-cineole (11.1%) were reported as main constituents [15]. Selina-5,11-diene (45.3%), δ-3-carene (7.8%), 1.8-cineole (4.2%) and β-caryophyllene (4.9%) were reported as main constituents of the aerial parts of the *H. forsskahlii* (Gmel) Hilliard et Burt oil from Saudi Arabia [17].

Essential oil from South African *H. dasyanthum* (Willd.) Sweet yielded 1,8-cineole (20.6%), α-pinene (16.6%) and β-caryophyllene (13.3%); *H. excisum* (Thunb.) Less: 1,8-cineole (34.0%), viridiflorol (18.2%); *H. felinum* Less: β-caryophyllene (27.6%), α-humulene (9.4%) and alloaromadendrene (7.3%) and *H. petiolare* Hilliard and Burtt: 1,8-cineole (22.4%), β-caryophyllene (14.0%) and *p*-cymene (9.8%) were reported as main constituents [16].

High abundance of fatty acids and their esters (24.9-70.8%) was detected in the herb volatiles of *H. plicatum* subsp. *polyphyllum* and *H. plicatum* subsp. *isauricum*. T-Cadinol (7.9%) was reported as main constituent of the aerial parts of the *Helichrysum plicatum* DC. subsp. *plicatum* from Turkey. The inflorescences of *Helichrysum* subspecies were found to be rich in monoterpenes (15.0-93.1%), fatty acids (0.1-36.3%) and sesquiterpenes (1.1-25.5%). The inflorescence volatiles of *H. plicatum* subsp. *isauricum* were distinguished by predomination of monoterpene hydrocarbons (93.1%) with fenchene (88.3%) as the major constituent [18].

The main components of the *H. conglobatum* oil from Cyprus were β-caryophyllene (14.6%), ɣ-curcumene (14.1%), hexadecanoic acid (13.5%) and tetradecanoic acid (7.5%) [19].

In our study, the main constituents were identified as hexadecanoic acid (25.3%), dodecanoic acid (17.8%), tetradecanoic acid (13.7%), and decanoic acid (4.2%) for *H. noeanum*. Decanoic acid (18.7%), tetradecanoic acid (16.9%), dodecanoic acid (13.8%), hexadecanoic acid (12.2%) and hexahydrofarnesyl acetone (3.2%) were found to be the major compounds for *H. chionophilum*. Elkiran et al. [20] have previously reported ɣ-Gurjunene (11.06%), spathulenol (9.90%), alloaromadendrene (7.53%) and β-caryophyllene (7.10%) as main constituents in the essential oil of *H. noeanum* from Ankara.

To the best of our knowledge, the essential oil of *H. chionophilum* has not chemically been investigated previously.

Acknowledgments

This study was financially supported by Anadolu University Scientific Research Projects Commission under the grant no: 1805S209.

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