

## Phytochemical composition of *Helichrysum arenarium* (L.) Moench essential oil (aerial parts) from Turkey

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### Abstract

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#### Keywords:

*Helichrysum  
arenarium* L.  
Oil  
Essential  
Phenolic  
Antioxidant

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#### Article history:

Received  
29.03.2020  
Received in  
revised form  
03.09.2020  
Accepted  
30.09.2020

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#### DOI:

10.24263/2304-  
974X-2020-9-3-3

**Introduction.** The aim of the present study was to determine the phytochemical composition of *Helichrysum arenarium* (L.) Moench (aerial parts) essential oil and extracts obtained from Turkey as a source of bioactive components for the food, cosmetics and pharmaceutical industry.

**Materials and methods.** The flower essential oil was obtained by hydrodistillation for 2 h and its composition was analyzed using gas chromatographic analysis (GC/MS). Extracts were obtained from the air-dried flowers by treatment with 70 and 90% ethanol (1:5 v/w) in an ultrasonic bath. In the extracts, the total phenolic content was obtained by Folin-Cicalteu method. The total flavonoid content was determined by Al(NO<sub>3</sub>)<sub>3</sub> reagents, measured at wavelength 415 nm against a blank. Antioxidant activity of the extracts was determined using DPPH, ABTS, FRAP, and CUPRAC assay.

**Results and discussion.** The amount of extracted essential oil was 0.07% with the main constituents oleic acid (30.28%), ethyl hexadecanoate (20.19%), linoleic acid (18.89%), and sclareol (4.22%). The composition of the oil predominated by oxygenated hydrocarbons (76.90%), followed by diterpenes (11.50%), hydrocarbons (3.53%), oxygenated sesquiterpenes (3.30%), phenyl propanoids (2.93%), sesquiterpene hydrocarbons (1.43%), monoterpene hydrocarbons (0.34%), and oxygenated monoterpenes (0.07%). The content of the total polyphenols in the 95% ethanol extracts was 7.56 mg GAE/g dw and the total flavonoids was 3.13 mg GAE/g dw, whereas their content in the 70% ethanol extract were 6.62 mg GAE/g dw and 3.34 mg GAE/g dw, respectively. Among the plant samples, 70% ethanol extract exhibited higher CUPRAC (159.46 mM TE/g dw) followed by ABTS radical action decolourization (47.53 mM TE/g dw). The results for ethanol extracts and their antioxidant activity were in accordance with the total phenolic content and the concentration of flavonoids. The flowers of *H. arenarium* were dominated by total carotenoid (10.68 µg/g dw), followed by total chlorophyll (51.24 µg/g dw). The content of chlorophyll a (32.67 µg/g dw) was higher than that of chlorophyll b (18.57 µg/g dw).

**Conclusions.** The main compounds of the *H. arenarium* essential oil were oleic acid, ethyl hexadecanoate, linoleic acid, and sclareol. The extracts showed a high level of total phenolic content and antioxidant potential, which make the studied plant species as a potential source of alternative natural antioxidant or a source of biologically active components.

## Introduction

*Helichrysum arenarium* L., commonly known as immortelle or everlasting among the population, is a plant with a pronounced potential for use and application in various spheres of life. The immortelle belongs to the genus *Helichrysum* (*Asteraceae* family) and includes over 600 species in the world. The genus is represented also in Turkish flora by 27 taxa (21 species and 6 subspecies), and 15 of them are endemic [1].

Due to the wide range of application of the plant, in some regions of the Turkey the plant was cultivated for the purpose of industrial production of essential oil, for use in cosmetics and food industry.

The species is widely used for essential oil isolation, because the oil have economic and pharmaceutical importance as they are used as a plant preservative [2]. The majority of authors confirm that the therapeutical effect of plants is attributed mainly to the presence of flavonoids and polyphenols [3].

In Turkey, the plant has been used only in folk medicine [1], which is a prerequisite for its cultivation for use in other sectors such as food, pharmacy and cosmetics industry.

The literature contains data on the chemical composition of the essential oil obtained from plants growing in different countries of Europe and Asia.

Radusiene and Judzentiene [4] isolated the essential oil from the differently colored (citric-yellow, citric, yellow, orange, yellow-brown and brown) inflorescences of *H. arenarium* growing in Lithuania. *Trans*-caryophyllene (8.8%),  $\delta$ -cadinene (8.2%), and 1,8-cineole (7.0%) were the major constituents in essential oils from citric inflorescences. The oil from brown inflorescences contained *trans*-caryophyllene (7.8%), whereas tetradecanoic acid (7.0%) was the major constituents of oils from yellow and yellow-brown inflorescences. The main constituents of *H. arenarium* essential oil from China were sesquiterpenes  $\beta$ -spatulanol (19.9–24.03%), ledol (6.22–10.02%), bicyclogermacrene (5.68%), aromadendrene (5.15%), and  $\alpha$ -eudesmol (4.29–4.37%) [5]. The chemical composition of *H. arenarium* essential oil from Hungary was represented by linalool (1.7%), anethole (3.2%), carvacrol (3.6%), and  $\alpha$ -muurolene (1.3%) [6].

The analysis of the data showed that the chemical composition of the essential oil was different, which depends on both the method of its obtaining and the soil and climatic conditions of growing the plant.

The *H. arenarium* essential oil demonstrated antimicrobial activity [7, 8] against various microorganisms, including pathogenic strains. Other authors, such as Tepe *et al.* [9] reported the antioxidant activity of the methanol extracts of four Turkish *Helichrysum* species.

Although the immprtelle plant was extensively investigated in different parts of the world, there were no data in the literature for the determination of secondary metabolites (pigments chlorophyll and carotenoids, essential oil, phenolic compounds and flavonoids) in the flowers of *H. arenarium* growing in Turkey, as well as for the antioxidant activity of its ethanolic extracts.

Therefore, the aim of this study was to determine the phytochemical composition of *Helichrysum arenarium* (L.) Moench (aerial parts) essential oil and extracts obtained from Turkey as a source of bioactive components for the food, cosmetics and pharmaceutical industry.

## Materials and methods

### Plant material

The aerial parts of *H. arenarium* were collected in full flowering stage on 31 July 2018 from Yozgat Akdagmadeni district (39°34.647' N 35°48.568' E, altitude 1760 m). Then dried at room temperature (18±2 °C) and relative humidity of 65% for 14 days. The samples were packed in plastic bags and stored at 23±2 °C until being analyzed.

### Isolation of essential oil

The moisture of the flowers (9.91%±0.09) was determined by drying up to constant weight at 105 °C [10].

The air-dried flowers were cut to a size of 0.5 mm. The essential oil was isolated by hydrodistillation for 2 h in a laboratory glass apparatus of the British Pharmacopoeia [11]. The oil obtained was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until analysis.

### Chemical composition of essential oil

The GC analysis was performed using a GC Agilent 7890A, an HP-5 ms column (30 m x 250 µm x 0.25 µm), temperature: 35 °C/3 min, 5 °C/min to 250 °C for 3 min, total: 49 min; helium as a carrier gas at 1 mL/min constant speed, and 30:1 split ratio.

The GC/MS analysis was carried out on an Agilent 5975C mass spectrometer, using helium as a carrier gas, and the same column and temperature as in the GC analysis.

The identification of chemical compounds was done by comparison to their relative retention time and library data. The identified constituents were arranged in order of retention time and quantity in percentage.

### Ultrasound-assisted extraction

The ultrasound-assisted extraction of biologically active substances from air-dried flowers was performed in an ultrasonic bath SIEL UST 5.7–150, Gabrovo, Bulgaria, with frequency 35 kHz and 300 W. The extraction procedure was performed with two solvents with different polarity 95% and 70% ethanol in the ratio (1:5 v/w). The dried plant materials were weighed in a 50 mL centrifuge tube with a screw cap and then 20 mL solvent was added to the sample. The tubes were placed in the ultrasonic bath at 55 °C for 20 min. The ultrasound-assisted extraction was performed in triplicate. Each extract was filtered and the combined extracts were used for further analysis.

### Total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent [12], as the extract (0.2 mL) was mixed with 1 mL Folin–Ciocalteu reagent diluted (1:5) and 0.8 mL 7.5% sodium carbonate. After 20 min at room temperature (25 °C) in darkness, the absorption was measured at wavelength 765 nm against a blank sample. [13].

### **Total flavonoids content**

The total flavonoids content was analyzed by Al (NO<sub>3</sub>)<sub>3</sub> reagents and measured at wavelength 415 nm against a blank [14]. The results were presented as mg quercetin equivalents (QE) per g dw.

### **Antioxidant activity**

The DPPH radical scavenging activities of flowers were evaluated [15], and the absorbance was measured at wavelength 517 nm. Radical scavenging activity of samples was expressed as mM Trolox® equivalent (TE) per g dw.

### **ABTS radical action decolourization assay**

ABTS radical was generated as an aliquot part of 7.0 mM 2,2'-azinobis(3-ethylbenzthiazoline)-sulfonic acid (ABTS, Sigma) in distilled water was mixed with 2.45 mM potassium persulfate in distilled water. The reaction was performed for 16 h at ambient temperature (25 °C) in darkness. Before analyses, 2.0 mL of generated ABTS<sup>+</sup> solution was diluted with methanol at proportions 1:30 (v/v), to the final absorbance of the working solution about 1.0 ÷ 1.1 at wavelength 734 nm. ABTS<sup>+</sup> solution (2.85 mL) was added to 0.15 mL extracts. After 15 min at 37 °C in darkness, the absorbance was measured at wavelength 734 nm against methanol. The antioxidant activity was expressed as mM TE/g dw [13].

### **Ferric reducing antioxidant power assay (FRAP)**

The FRAP method was performed as previously described [16] and the absorbance was recorded at wavelength 593 nm. The results were expressed as mM Trolox® equivalent per g dw.

### **Cupric reducing antioxidant capacity (CUPRAC) assay**

One mL CuCl<sub>2</sub> × 2H<sub>2</sub>O, 1 mL Neocuproine (7.5 mL in methanol), 0.1 M ammonium acetate buffer (1 mL), 0.1 mL extracts and 1 mL distilled water was mixed. The reaction was performed for 20 min at 50 °C in darkness. After cooling the absorbance was measured at wavelength 450 nm. The antioxidant activity was expressed as mM TE/g dw [13].

### **Total Chlorophylls and Carotenoid content**

For determination of chlorophyll a, chlorophyll b, total chlorophylls and the total carotenoids, the absorbance of 95% ethanol extracts was measured at three wavelengths 664 nm, 648 nm, and 470 nm. The amount of these pigments was calculated according to the formulas (–4), [17].

$$\text{Chlorophyll a (Ca)} = 13.36A_{664.2} - 5.19A_{648.6} \quad (1)$$

$$\text{Chlorophyll b (Cb)} = 27.43A_{648.6} - 8.12A_{664.2} \quad (2)$$

$$\text{Total Chlorophyll (a+b)} = 5.24A_{664.2} + 22.24A_{648.6} \quad (3)$$

$$\text{Total carotenoids} = [1000A_{470} - 2.13C_a - 97.64C_b]/209 \quad (4)$$

## Results and discussion

### Chemical composition of essential oil

In our study, the essential oil content (yield) was  $0.07\% \pm 0.00$  in the plants. The chemical composition of *H. arenarium* essential oil was presented in Table 1.

In the essential oil, 42 constituents representing 97.89% of the total content were identified. Ten of them were in concentrations over 1% and the rest 32 constituents were in concentrations under 1%. The main constituents in the plant essential oil (above 3%) were: oleic acid (30.28%), ethyl hexadecanoate (20.19%), linoleic acid (18.89%), and sclareol (4.22%).

The main components' groups were found to be oxygenated hydrocarbons (oleic acid, ethyl hexadecanoate, and linoleic acid), followed by diterpenes (sclareol), hydrocarbons (n-tetracosane), oxygenated sesquiterpenes (cedrol), phenylpropanoids (carvacrol), sesquiterpene hydrocarbons, monoterpene hydrocarbons, and oxygenated monoterpenes.

The chemical composition of *H. arenarium* essential oil was previously studied by several researchers [18, 19, 20]. In their paper, Leonardi et al. [18] analyzed essential oils obtained from six species of *H. arenarium* growing in Italy. One hundred seventeen components were identified representing 90.1–98.5% of the total chemical composition, mainly characterized by sesquiterpenes and monoterpene compounds. Judzentiene and Butkiene [19] studied the essential oil composition of *H. arenarium* of leaves and inflorescences of different colors from natural populations from eastern Lithuania. It was found that the principal constituents were  $\beta$ -caryophyllene (in three inflorescences and one leaf oil),  $\delta$ -cadinene (in two leaf samples), octadecane (in one leaf sample) and heneicosane (in one inflorescence sample). Later, in 2019, Judzentiene et al. [20] investigated the chemical composition of the 16 essential oils obtained by hydrodistillation from inflorescences and leaves of *H. arenarium* plants growing in Lithuanian forests. Similarly to the data reported by Leonardi et al. [18], they determined that the main fractions were found to be sesquiterpenes (29.0–70.1%) and aliphatics hydrocarbons (7.7–45.5%). It is also reported that  $\beta$ -caryophyllene,  $\delta$ -cadinene, and octadecane, were among the main constituent of *H. arenarium* essential oil collected in Lithuania. Different species of *Helichrysum* showed significant differences in chromatographic profiles. The results reported by Juliano et al. [21] showed that the chemical composition of *H. microphyllum* subsp. *tyrrhenicum* essential oils collected in four different regions in South-Western Sardinia, was represented by a significant amount of neryl acetate,  $\gamma$ -curcumene, and linalool. Kurkuoglu et al. [22] reported a study for the chemical composition, simultaneously analyzed by GC/FID and GC/MS obtained by the aerial parts of *H. noeanum* Boiss. and *H. chionophilum* Boiss. et Balansa. They reported that the main constituents were identified as hexadecanoic acid, dodecanoic acid, tetradecanoic acid, and decanoic acid. Besides the specificity of studied species the differences between the essential oil composition in this study and that from other studies, reported in the literature, are probably due to the climatic conditions in the respective location where the plants were grown, and also to the plant parts processed and obtained.

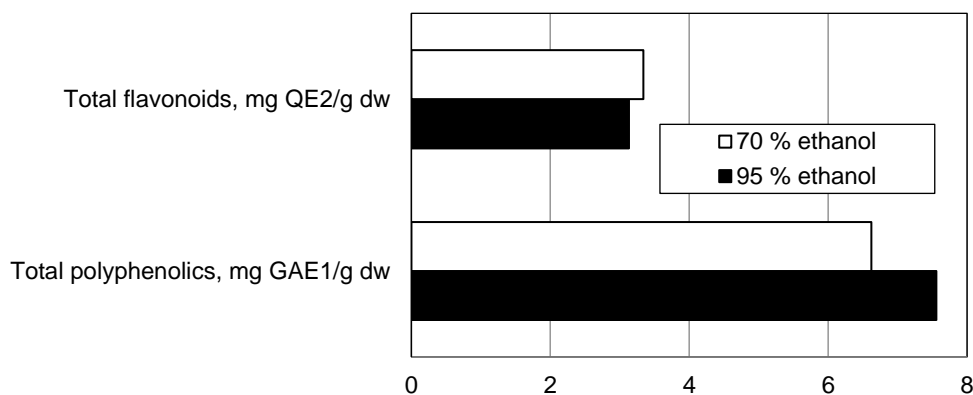
Table 1

Chemical composition of the *H. arenarium* essential oil

№	Name	RI	Content, %
1.	$\alpha$ -Pinene	932	0.10 ± 0.0
2.	<i>p</i> -Cymene	1019	0.08 ± 0.0
3.	$\gamma$ -Terpinene	1054	0.07 ± 0.0
4.	$\beta$ -Linalool	1095	0.07 ± 0.0
5.	Borneol	1165	0.10 ± 0.0
6.	n-Dodecane	1200	0.06 ± 0.0
7.	Thymol	1289	0.11 ± 0.0
8.	Carvacrol	1297	2.68 ± 0.02
9.	$\beta$ -Caryophyllene	1417	0.56 ± 0.0
10.	Aromadendrene	1439	0.17 ± 0.0
11.	$\alpha$ -Caryophyllene	1453	0.13 ± 0.0
12.	Germacrene D	1484	0.11 ± 0.0
13.	$\gamma$ -Cadinene	1513	0.23 ± 0.0
14.	$\delta$ -Cadinene	1522	0.20 ± 0.0
15.	(2E)-Tridecenol	1568	0.10 ± 0.0
16.	Spathulenol	1576	0.15 ± 0.0
17.	Caryophyllene oxide	1582	0.13 ± 0.0
18.	Viridiflorol	1591	0.21 ± 0.0
19.	Cedrol	1600	0.67 ± 0.0
20.	$\beta$ -Cedren-9-one	1632	0.08 ± 0.0
21.	epi- $\alpha$ -Cadinol	1640	0.48 ± 0.0
22.	$\alpha$ -Cadinol	1653	0.20 ± 0.0
23.	7-epi- $\alpha$ -Eudesmol	1662	0.32 ± 0.0
24.	$\alpha$ -Bisabolol	1685	0.10 ± 0.0
25.	Cedroxide	1713	0.14 ± 0.0
26.	Methyl tetradecanoate	1722	2.79 ± 0.02
27.	(2E,6E)-Farnesyl acetate	1844	0.31 ± 0.0
28.	n-Nonadecane	1900	0.26 ± 0.0
29.	Methyl hexadecanoate	1922	2.54 ± 0.02
30.	Phytol	1943	0.20 ± 0.0
31.	Sclarene	1974	0.30 ± 0.0
32.	Ethyl hexadecanoate	1997	20.19 ± 0.19
33.	n-Eicosane	2000	0.16 ± 0.0
34.	n-Heneicosane	2100	0.18 ± 0.0
35.	Methyl octadecanoate	2124	0.48 ± 0.0
36.	Linoleic acid	2132	18.89 ± 0.17
37.	Oleic acid	2141	30.28 ± 0.29
38.	Sclareol	2222	4.22 ± 0.04
39.	3 $\alpha$ -hydroxy-Manool	2301	0.67 ± 0.0
40.	3 $\alpha$ -14,15-dihydro-Manool oxide	2337	2.89 ± 0.02
41.	3 $\alpha$ -acetoxo-Manool	2362	2.98 ± 0.02
42.	n-Tetracosane	2400	2.86 ± 0.02
		Hydrocarbons, %	3.53
		Oxygenated hydrocarbons, %	76.90
		Monoterpene hydrocarbons, %	0.34
		Oxygenated monoterpenes, %	0.07
		Sesquiterpene hydrocarbons, %	1.43
		Oxygenated sesquiterpenes, %	3.30
		Phenyl propanoids, %	2.93
		Diterpenes, %	11.50

### Total phenolic and flavonoid content

The results for total phenolic and flavonoids content of *H. arenarium* extracts are shown in Figure 1. The total phenolic content of the 95% ethanol extracts was higher ( $7.56 \pm 0.08$  mg GAE/g dw) than the other sample. On the other hand, the amount of flavonoids for 70% ethanol was greater ( $3.34 \pm 0.15$  QE/g dw) than that of 95% ethanol extract. Our results for 70% ethanol extracts were lower than the results obtained by Babota et al. [23] for the phenolic content (25.17 mg GAE/g dw) and total flavonoid content (27.17 QE/g dw). In a study performed by Albayrak et al. [24] the TPC value ranged from 71.81 to 125.57 mg GAE/g of methanolic extracts of two subspecies of *H. arenarium* from Turkey.



\*<sup>1</sup>GAE - Gallic Acid Equivalents, <sup>2</sup>QE - Quercetins Equivalents

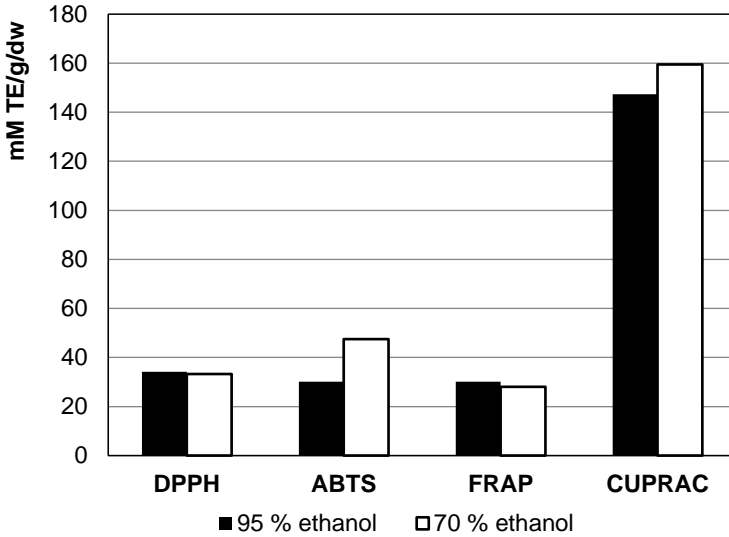
**Figure 1. Total polyphenolics and flavonoids content of *H. arenarium* extracts.**

### Antioxidant activity

The antioxidant activity of *H. arenarium* extracts, determined via four methods (DPPH, ABTS, FRAP, and CUPRAC) was presented in Figure 2. The anti-radical activity of the samples with 70% ethanol displayed higher values than the samples with 95% ethanol by the methods of ABTS and CUPRAC.

Our results were higher than that reported by Babota et al. [23] who determined the antioxidant activity of 70% ethanol extracts of *H. arenarium* against the stable synthetic ABTS radical action ( $5.82 \pm 0.02$  mM TE/g/dw) and the method of DPPH ( $17.88 \pm 7.20$  mM TE/g/dw). Albayrak et al. [24] reported lower values for antioxidant activity (23.03 mM TE/g/dw and 47.64 mM TE/g/dw) determined by the method of DPPH for two subspecies of *H. arenarium* from Turkey.

The results for ethanol extracts and their antioxidant activity were in accordance with the total phenolic content and the concentration of flavonoids.



**Figure 2. Antioxidant activity of *H. arenarium* extracts**

### Total Chlorophylls and Carotenoid Content

The chlorophyll and carotenoid contents in the analysed samples were presented in Table 2. Ethanol is a solvent that dissolves both polar (hydrophilic) and non-polar (hydrophobic/lipophilic) substances. The results showed that the total chlorophyll content predominated in the composition of the sample, followed by carotenoid content.

**Table 2**

**Content of pigments in the dry raw material of *H. arenarium*, µg/g dw**

<i>H. arenarium</i>	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Total Carotenoid
	32.67±0.21	18.57±0.08	51.24±0.24	10.68±0.11

According to previous reports [17], the number of porphyrin pigments varied depending on environmental conditions, high-temperature inversions, lack of water and minerals. Higher levels of chlorophyll a (32.67%) than those of chlorophyll b (18.57%) could be a marker of the presence of contamination [25] in both the soil layers and the air where the samples were collected. The extraction method of carotenoids was similar to that of chlorophylls, as they are part of the photosynthetic pigments in plant cells. The values of total chlorophylls and carotenoids in the tested samples may vary depending on the type of solvent used [17], its polarity and the conditions of their extraction. Changes in the content of chlorophyll pigments were a typical abiotic stress associated primarily with induced aging, light stress, nitrogen deficiency and others, in which initially the content of chlorophyll a decreased stronger than chlorophyll b.



## Conclusions

1. The qualitative composition of the obtained essential oil from *H. arenarium* from Turkey demonstrated the content of volatile biologically active components with potential for use in the food, cosmetics and pharmaceutical industry,
2. The ethanol extracts from *H. arenarium* from Turkey exhibited pronounced antioxidant properties based on high levels of flavonoids, polyphenols and porphyrin pigments.

**Acknowledgements.** This work is part of a project supported by the Yozgat Bozok University Scientific Research Projects Unit (BAP; Project Code: 6602a-TBMYO/2–356, Yozgat, Turkey).

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