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The determination of some biochemical contents of *Helichrysum armenium* DC. subsp. *araxinum* (Kirp.) Takht

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Abstract

The aim of current study was to determine fatty acid compositions, lipid soluble vitamins, phenolic compounds, radical scavenging activities, antioxidant activity and metal chelating capacity of *Helichrysum armenium* subsp. *araxinum* extracts. It was found that palmitic acid (C16:0) was primary fatty acid (58,99±1,21%). Current study demonstrated that lipid soluble vitamin contents were lowest. However, stigmasterol and β-sitosterol were found as 79,4±1,17 and 29,2±0,87, respectively in this study. Also, present study showed that *H. armenium* subsp. *araxinum* has highest naringin content (1156,8±3,57 µg/g) whilst it has high vanillic acid (213,2±2,32 µg/g), caffeic acid (151,6±1,27 µg/g) and ferulic acid (117,94±1,26 µg/g) contents. On the other hand, it was demonstrated that *H. armenium* subsp. *araxinum* has highest DPPH (except for 10 µl), ABTS radical scavenging activities and metal chelating capacity in 100 µl.

Key words: fatty acids, *Helichrysum armenium* subsp. *araxinum,* lipid soluble vitamins, phenolics, radical scaveging activity

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Helichrysum armenium DC. subsp. araxinum (Kirp.) Takht. taksonunun bazı biyokimyasal içeriklerinin belirlenmesi

Özet

Bu çalışmanın amacı, *Helichrysum armenium* subsp. *araxinum* özütlerinin yağ asidi bileşimi, yağda çözünen vitaminleri, fenolik bileşikleri, radikal temizleme faaliyetleri, antioksidan aktivitesi ve metal şelatlama kapasitesini belirlemektir. Araştırmada palmitik asidin (C16:0) birincil yağ asidi olduğu ve (58,99±1,21%) yağda çözünen vitamin içerilerinin en düşük seviyede olduğunu belirlenmiştir. Stigmasterol ve β-sitosterol, sırasıyla 79,4 ± 1,17 ve 29,2 ± 0,87 olarak tespit edilmiştir. Ayrıca mevcut çalışma, *Helichrysum armenium* subsp. *araxinum*'un en yüksek naringin içeriğine (1156,8 ± 3,57 µg / g) sahip olduğunu ayrıca yüksek miktarda vanilik asit (213,2 ± 2,32 µg / g), kafeik asit (151,6 ± 1,27 µg / g) ve ferulik asit (117,94 ± 1,26 µg / g) içeriğine sahip olduğunu göstermiştir. Öte yandan, *Helichrysum armenium* subsp. *araxinum*, en yüksek DPPH (10 µl hariç), ABTS radikal temizleme aktivitesi ve 100 µl'de metal şelatlama kapasitesine sahiptir.

Anahtar kelimeler: yağ asitleri, *Helichrysum armenium* subsp. *araxinum,* yağda çözünen vitaminler, fenolikler, radikal temizleme aktivitesi

1. Introduction

The name of *Helichrysum,* is composed of the combination of Greek words helios and chryos which meaning sun and gold because it has inflorescences of a shining yellow [1]. The genus is usually known as "Altın Otu" or "Ölmez

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Çiçek" in Turkey and used as herbal tea [2]. The genus comprises more than a thousand taxa which significantly spread out in the Mediterranean areas. Genus Helichrysum is naturally distributed in Turkey [3-6]. In Turkish flora, the genus is represented by 27 taxa, of which the endemism ratio is 55 % [7]. The members of genus may be annuals, herbaceous perennials or shrubs, expanding to a height of 90 cm [8]. It grows on rocky, dry or sandy areas in the Mediterranean [9].

Species from *Helichrysum* generally are used traditional medicine against kidney stones, inflammation, gallbladder, cold, liver, diabetes and digestive disorders [10,11]. It has been approved by World Health Organization and European Medicines Agency consuming as the infusion or decocation of flowers of species from genus [12]. It is accepted that the curative effects of genus is chiefly due to some antioxidants including mainly flavonoids, coumarins, phenolic acids contents. Phenolics have significant role as antioxidant against lipid peroxidation [13]. Their antioxidant capacity is come from act as hydrogen donor, scavenging free superoxide cavenger and metal chelating [14]. The aim of present study is to determine the fatty acids, lipid soluble vitamins, sterols, phenolic contents, radical scavenging activities, metal chelating of *Helichrysum armenium* subsp. *araxinum*.

2. Materials and methods

2.1. Chemical agents

All chemicals were supplied from Sigma-Aldrich.

2.2. Plant materials

Sample plants were collected from Harput/Elazig and identified by Dr. Murat KURSAT in 2011 and were stored in the Firat University Herbarium. The fatty acid compositions, lipid soluble vitamins, flavonoids, phenolic acids radical scavenging activity (DPPH and ABTS), Fe chelating were evaluated in the *Helichrysum armenium* subsp. *araxinum* extracts. Experiments were repeated three times.

2.3. The analysis of fatty acid, lipid soluble vitamins and sterol

2 g seed materials finely ground in a mill and were extracted with isopropanol/hexane (2:3 v/v) for fatty acid, sterol and vitamin analyses were [15]. The lipid extracts were centrifuged at 10.000 g for 5 minutes and filtered. The solvent was then removed by using rotary evaporator at 40°C. The samples were kept at -25°C. The experiment was repeated three times.

2.3.1. The analysis of fatty acids

2% sulphuric acid (v/v) in methanol was used to form fatty acid methyl esters [16]. The fatty acid methyl esters were treated with n-hexane and separated by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to a Glass GC 10 software. Nitrogen was used as carrier gas flow ratio 0.8 ml/min. and capillary column (25 m in length and 0.25 mm in diameter; Permabound 25, Macherey-Nagel, Germany) was used to conduct the chromatographic analysis. The heat of, detector, column and injection valve were adjusted as 240, 130-220, and 280°C, respectively.

2.3.2. Chromatographic analysis and quantification of lipid soluble vitamins and sterols

Lipid-soluble vitamins and phytosterols were taken from the lipid fraction based on the method of Sánchez-Machado [17]. The samples were dissolved in acetonitrile/methanol (75/25 v/v) and 50 mL were injected into the HPLC (Shimadzu, Japan). A Supelcosil TM LC18 (250 x 4.6 mm, 5 mm, Sigma, USA) was used as column and acetonitrile/methanol (75/25, v/v) was used as mobile phase. The temperature of the column was kept at 40°C. Determination was performed at 320 nm for retinol (vitamin A) and retinol acetate, and 215 nm for d-tocopherol, vitamin D, a-tocopherol, a-tocopherol acetate, 202 nm for phytosterols, 265 nm for vitamin K1 [18]. Class Vp 6.1 software was used to obtain data. The results of the analyses were written as µg/g for samples.

2.4. Extraction of seed materials for phenolics

Homogenisation was conducted by using 5 ml 80% methanol to measure the flavonoid and phenolic acids. The samples were centrifuged at 5000 rpm at +4ºC and dimethyl sulphoxide (DMSO) was used to provide a reserve solution.

2.5. Chromatographic conditions for flavonoids

Chromatographic analysis was done method offered by Zu et al. [19]. Column was PREVAIL C18 reversed-phase column (15x4.6mm, 5µm, USA) and mobile phase was methanol /water/acetonitrile (46/46/8, v/v/v) comprisng 1.0% acetic acid [19]. It was used 1.0 ml/min. as flow ratio and chromatographic peaks were confirmed by determining

retention times with those of the standards. Resveratrol, quercetin, naringenin, naringin, catechin, myricetin, morin, rutin, kaempferol and vanillic acid, cinnamic acid, caffeic acid, ferulic acid and rosmarinic acid were measured by DAD following RP-HPLC. Flow ratio were 1.0 ml/min and the chromatographic peaks of the samples were affirmed by measuring retention times with those of the standards. Chromatographic studies were done at 25°C.

2.6. Antioxidant activity

2.6.1. DPPH radical scavenging capacity

DPPH radical was afresh prepared based on method by Liyana- Pathiranan and Shahidi (2005). 4. 0 ml DPPH solution were mixed with 25, 50, 100, 150 and 250 µL of extract. The complex were kept at the dark for 30 minutes at room temperature. The absorbances were measured spectrophometrically at 517 nm. 1 µM quercetin was used as reference [20]. The results were determined by using formula:

DPPH radical scavenging capacity (%) = [(Abs_control – Abs_sample)]/(Abs control)] x 100

Abs_control is the absorbance of DPPH radical + methanol; Abs_sample is the absorbance of DPPH radical + sample extract/standard.

2.6.2. ABTS 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assay

ABTS radical cation assay was defined method done by Ree et al. [21]. The ABTS radical cation (ABTS•+) and 7 mM ABTS mixed with 2.45 mM potassium persulphate to obtain ABTS radical cation. The solution kepted for 12–16 h at room temperature. The (ABTS•$^{+}$) solution was dissolved with water to measure an absorbance of 0.700 ± 0.020 at 734 nm. 3 ml ABTS solution were mixed with 25, 50, 100, 150 and 250 µL of extract and absorption was detected during 6 min. Absorbance of control (3.0 mL (ABTS•+) solution with 30 L water) was written as Acontrol [22].

ABTS radical cation scavenging capacity (%) = [(Abs_control – Abs_sample)]/ (Abs_control)] x 100

2.6.3. Metal chelating activity

The chelating capacity were done method by Dinis et al. [23]. 50 µl of 2 mM $FeCl_2$ was added to several concentrations including 50, 100, 250 and 500 µg/mL of extracts. 5 mM ferrozine (0.2 mL) added to mixture to start reaction. The solution kept at the room temperature for 10 minutes after the solutions was mixed strongly. 562 nm was used as abosrbance [23]. The percentage inhibiting of of ferrozine–Fe^{2+} complex was found following formula:

Ferrous ion chelating activity (%) = [1 - (As /Ac)] × 100 where A_c was the absorbance of the control, and As was the absorbance of the extract/ standard [24]. Positive control was Na_2EDTA.

3. Results

Table 1. Fatty acid compositions of *Helichrysum armenium* subsp. *araxinum*

Fatty Acids	Fatty acid composition %
10:0	1,88±0,27
14:0	2,72±0,32
16:0	58,99±1,21
16:1 n7	4,73±0,33
18:0	5,47±0,74
18:1 n9	9,20±0,84
18:2 n6c	5,57±0,68
18:3n3	2,65±0,2
24:1	8,74±0,79

Present study showed that *Helichrysum armenium* subsp. *araxinum* has highest palmitic acid content (C16:0; 8,99±1,21%). The other saturated fatty acids were stearic acid (C18:0; 5,47±0,74%), capric acid (C10:0; 1,88±0,27%) and myristic acid (C14:0; 2,72±0,32%). On the other hand, oleic acid (18:1 n9; 9,20±0,84%), linoleic (C18:2 n 6; 5,57±0,68), linolenic acids (C18:3 n 3; 2,65±0,2) and nervonic acid (C:24:1; 8,74±0,79%) were found as unsaturated fatty acids.

Table 2. Lipid soluble vitamin and sterol contents of *Helichrysum armenium* subsp. *araxinum*

Lipid-soluble vitamins	Amounts (µg/g)
K1	0,1±0,01
K2	0,1±0,01
R-tocopherol	0,4±0,01
D2	0,3±0,01
D3	0,05±0,01
a-tocopherol	4,5±0,57
Ergosterol	0,5±0,01
Stigmasterol	79,4±1,17
B-sitosterol	29,2±0,87

It was found that lipid soluble vitamin and ergosterol contents of *Helichyrsum* were lowest or trace amounts. However, stigmasterol (79,4±1,17 µg/g) and β-sitosterol (29,2±0,87 µg/g) contents of *Helichrysum armenium* subsp. *araxinum* were relatively high.

Table 3. Flavanoid contents of *Helichrysum armenium* subsp. *araxinum*

Flavonoids	Amounts (µg/g)
Rutin	396,24±2,57
Myricetin	18,8±0,59
Morin	1,1±0,04
Quercetin	0,2±0,01
Kaempherol	-
Catechin	181,8±2,47
Naringin	-
Naringenin	1156,8±3,57
Resveratrol	-

Present study showed that quercetin, kaempherol, naringenin and resveratrol contents of *Helichyrsum* were lowest or absent. However, the naringin amount of *Helichyrsum armenium* subsp. *araxinum* was found highest (1156,8±3,57 µg/g). Also, it was found that rutin (396,24±2,57 µg/g) and catechin (181,8±2,47 µg/g) amounts of *Helichyrsum armenium* subsp. *araxinum* were high. The myricetin content of *Helichyrsum armenium* subsp. *araxinum* was determined as 18,8±0,59 µg/g.

Table 4. Phenolic acid contents of *Helichrysum armenium* subsp. *araxinum*

Phenolic acids	Amount (µg/g)
Vanillic acid	213,2±2,32
Cinnamic acid	7,1±0,84
Caffeic acid	151,6±1,27
Ferulic acid	117,94±1,26
Rosmarinic acid	26,6±0,87

It was found that *Helichyrsum* has low cinnamic acid (7,1±0,84 µg/g) and rosmarinic acid contents (26,6±0,87 µg/g). However, it has high vanillic acid (213,2±2,32 µg/g), caffeic acid (151,6±1,27 µg/g) and ferulic acid (117,94±1,26 µg/g) contents.

Table 5. DPPH and ABTS radical scavenging activities of *Helichrysum armenium* subsp. *araxinum*

Concentrations	DPPH %	ABTS %
10 µl	36,1±0,97	98,1±1,11
25 µl	69,6±0,74	99,48±1,21
50 µl	92,4±1,1	98,62±0,97
100 µl	93,1±1,3	98,31±0,84
250 µl	92,2±0,94	98,96±0,79

Current study demonstrated that *Helichyrsum* has high DPPH radical scavenging activity except for 10 µl concentration. Also, this study showed that *Helichrysum armenium* subsp. *araxinum* has highest ABTS radical scavenging activity in all of concentrations studied.

Table 6. Fe Chelating activity of *Helichrysum armenium* subsp. *araxinum*

Concentrations	Fe-Chelating %
100 µl	93,2±1,14
500 µl	38,1±0,57

This study showed that *Helichrysum armenium* subsp. *araxinum* has 93,2±1,14% in 100 µl and 38,1±0,57% in 500 µl Fe-chelating activity.

4. Conclusions and discussion

It was found that *Helichrysum armenium* subsp. *araxinum* has highest saturated fatty acid content (69.06%). Palmitic acid (C16:0) was major saturated fatty acid (58,99±1,21%) whilst oleic acid (C18:1 n9) was main unsaturated fatty acid content of *Helichrysum armenium* subsp. *araxinum* (9,20±0,84%). Dilika et al. [25] indicated that *Helichrysum*

has linoleic and oleic acids contents. Also, Powell et al. [26] indicated that *Helichrysum* has usual palmitic, stearic, oleic, and linoleic acids. It was found that the lipid soluble vitamin contents of *Helichrysum armenium* subsp. *araxinum* were trace or lowest in the present study. In addition, present study showed that stigmasterol content was 79,4±1,17 µg/g and β-sitosterol was 29,2±0,87 µg/g, whilst ergosterol content was 0,5±0,01 µg/g. Different studies showed that *Helichrysum* has β-sitosterol and stigmasterol compounds [27]. As far as we know this is the first report for lipid soluble vitamin contents of *Helichrysum armenium* subsp. *araxinum.*

Present study showed that *Helichrysum armenium* subsp. *araxinum* has highest naringenin content (1156,8±3,57 µg/g). Also, this study found that rutin (396,24±2,57 µg/g) and catechin (181,8±2,47) contents of *Helichrysum armenium* subsp. *araxinum* were high. Pljevljakusic et al. [28] showed that *Helichrysum* has flavanones naringenin (4) and naringenin-5-O-glucoside are the dominant compounds in sandy everlasting. Also, Kolayli et al. [29] found that *Helichyrsum* has rutin, catechin and quercetin. However Albayrak et al. [14] suggested that *Helichrysum* doesn't contain rutin. They indicated that chlorogenic acid, apigenin-7-glucoside and apigenin contents of *Helychrysum* were highest [14]. It was demonstrated that apigenin, naringenin, apigenin-7-O-glucoside and naringenin-O-hexosides were major constituents study done Gradinaru et al. [30]. The literatures demonstrated that *Helichrysum* has apigenin, isorhamnetin, quercetin, myricetin, naringenin and kaempherol [31,11]. On the other hand, present study showed that *Helichrysum* has high vanilic acid (213,2±2,32 µg/g), caffeic acid (151,6±1,27 µg/g) and ferulic acid (117,94±1,26 µg/g). Also, it was found that *Helichrysum armenium* subsp. *araxinum* has cinnamic acid (7,1±0,84 µg/g) and rosmarinic acid (26,6±0,87 µg/g). Kolayli et al. [29] found that *Helichrysum* has vanilic acid, caffeic acid, cinnamic acid, chlorogenic acid, syringic acid, coumaric acid, ferulic acid, benozic acid and gallic acid. Also, Bryksa-Godzisz et al. [31] showed that high chlorogenic and ferulic acid contents. Furthermore, another study showed that *Helichrysum* has caffeic acid, chlorogenic acid and quinic acid [32].

This study showed that DPPH scaveging activity of *Helichrysum armenium* subsp. *araxinum* was high (apart from 10 µl**)** and *Helichrysum armenium* subsp. *araxinum* has highest ABTS radical scavenging acitivities in all of concentrations studied. Albayrak et al. [14] indicated that all the extracts of *Helichrysum* including *Helichrysum armenium* displayed an rising in free radical scavenging capacity. Aiyegoro and Okoh [8] concluded that *Helichrysum* has potently active DPPH and ABTS scavenging. Also, Gouveia and Castillo [33] demonstrated that all of *Helichrysum* extracts represented scavenging activity against DPPH and ABTS. Similarly, Aiyegoro and Okoho [34] indicated that *Helichrysum* extracts displayed scavenging activity against all radicals including DPPH and ABTS tested. Also, it was suggested that *Helichrysum* taxa represented strong free radical-scavenging activity [35,36]. On the other hand, this study showed that *Helichrysum armenium* subsp. *araxinum* has 93,2±1,14% in 100 µl and 38,1±0,57% in 500 µl Fe-chelating capacity. Haddouchi et al. [37] found that *Helichrysum* has highest ferric-ion chelating capacity. Similarly, the results of study done by Aiyegoro and Okoho [30] suggested that *Helichrysum* has Fe-chelating reducing power.

Present study demonstrated that saturated fatty acid composition of *Helychrysum armenium* subsp. *araxinum* highly especially palmitic acid and it has lowest lipid soluble vitamin contents. However, it was showed that *Helychrysum armenium* subsp. *araxinum* has highest naringin content and high vanilic acid, caffeic acid and ferulic acids. Moreover, current study suggested that *Helichrysum armenium* subsp. *araxinum* has strong antioxidant capacity.

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