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RESEARCH ARTICLE

EFFECT OF ALTITUDE ON FATTYACIDCOMPOSITION OF SEED OF WILD *ACHILLEA WILHELMSII* C. KOCH. SUBSP. *WILHELMSII* FROMTURKEY

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ABSTRACT

In this study, it has been aimed to determine the effect of altitude on fatty acid composition of seed oil of wild *Achillea wilhelmsii* from Turkey's flora. The fatty acid compositions were determined by gas chromatography (GC). The total lipid contents of seed (at altitude 1000-1080 m, 1600-1650 m and 1850-1900 m) were found to be 4.17, 4.27, and 2.73%, respectively. The lowest total lipid level (2.73%) was 1850-1900 m and significant as statistical. Total saturated (∑SFA), monounsaturated (∑MUFA) and polyunsaturated (∑PUFA) acid contents of seed oils at different altitude were significant difference. But fatty acid profiles were quite similar. According to the obtained results, ∑PUFA ω-6 significantly decreased and ∑PUFA ω-3 significantly increased with altitude. The major components of *A. wilhelmsii* seed oil are palmitic (C16:0), oleic (C18:1 ω-9) and linoleic (18:2 ω-6) acids. C16:0 and C18:1 ω-9 contents increased with altitude but 18:2 ω-6 content decreased with altitude. The quantities of other fatty acids ranged at different altitude in the terms of increase or decrease as statistical. The ω-6/ω-3 ratios were 67.28% at 1000-1080 m, 17.84% at 1600-1650 m and 9.53% at 1850-1900 m.

Key words: *Achillea Wilhelmsii*, Altitude, Fatty Acids, Seed Oil.

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INTRODUCTION

Achillea species are widely used in folk medicine for the preparation on herbal teas with antiphlogostic and spasmolytic activitiy and also they have been used an anti-inflammatory agents for treatment of rheumatic pains (Wichtl 1994). Therefore recently, plants and their extracts have been used in alternative medicine and society to treat various diseases (Howes *et al.,* 2003; Saeidnia *et al.,* 2011; Ertaş *et al.,* 2014). Some studies such as on biological activities, chemical composition, antiolytic evalution and essential oil effects on rat's gastric acid have been conducted on *Achillea wilhelmsii* (Niazmand *et al.,* 2010; Başer 2016; Çakır *et al.,* 2016). The features of plant and animal oils depends on the rate and composition of their contain fatty acids. Therefore, determination of fatty acid compositions of the oil will allow for production according to their in tented use. Variations in lipid and fatty acid composition between and within plant species depending on environmental factors such as temperature, season, location and altitude (Lajara et al.1990; Pritchard *et al.,* 2000; McCartney *et al.,* 2004; Harris *et al.,* 2006; Beyhan *et al.,* 2011). In spite of many works on the chemical constituents of some *Achillea* species, there is not sufficient data on the fatty acid compositions of this genus. The most of the studies on *Achillea* species were conducted on their essential oil composition (Aghjani *et al.,* 2000; Toncer *et al.,* 2010; Taherkhani *et al.,* 2012). Specially ω-6 and ω-3 fatty acids were also known to confer cardiac-health properties to human and the consumption of these fatty acids are recommend (Simopoulos 2002; Holup and Holup 2004; Land 2005). Consequentyl, the fatty acid dynamics of *Achillea* genus is not well known, and this work aims to establish the effect of altitude on fatty acids and determine the fatty acid composition of seed oil of *Achillea wilhemsii* from Turkey flora.

MATERIALS AND METHODS

Plant material: *Achillea wilhelmsii* C. Koch. subsp. *wilhelmsii* examples (from Turkish flora) used in this study, collected from C5 Niğde region (Maden village environment) in Aug at 2012 (temperature 22-23 ºC, altitude about 1000-1080 m), B6

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Kayseri region (between Kaftangiyen and Taşgeçit villages) in July 2012 (temperature 22-23 ºC, altitude 1600-1650 m) and B6 Sivas region (Imranlı-Karacaören, Bahadun crossroads) in June 2012 (temperature 19-20 ºC, altitude 1850-1900 m). This species material is stored at the herbarium of Biology Department of Cumhuriyet University.

Seeds extraction and fatty acids analysis: The air-dried seed materials at room temperature were ground. 3 g was taken from each of the milled seed material, and stored in chloroform-methanol (2/1, v/v) for 48 h at 4 ºC. The seeds were extracted in chloroform-methanol (2/1, v/v) on the ice bath. The isolation of the total lipid from seed samples were carried out (Folch *et al.,* 1957). The total lipids obtained were saponified by reflusing with methanol (50%) containing 6% potassium hydroxide for 1 h at 85 ºC. The saponifiable lipids were converted to fatty acid methyl esters (FAMEs) for 10 min at 85 ºC using the standard Boron triflouride-methanol (BF3) method (Moss *et al.,* 1974).

Gas chromatography (GC) analyses: The resultant mixture of FAMEs in hexane:chloroform (4/1, v/v) was injected into HP (Hewlett Packard) Agilent 6890N model GC equipped with a flame ionization detector (FID), and fitted with an HP-88 capillary column (100m x 0.20mm i.d., 0.25 µm film). The carrier gas was helium (1 mL min-1) and injector port and detector temperatures were 240 and 250 ºC, respectively. A small quantity of FAMEs solution (1 µl) was introduced onto the column. Column temperature program was 160 ºC for the beginning, then increasing at 4 ºC/min up to 185 ºC and then increased 1 ºC/min up to 200 ºC. Identification of normal fatty acids was carried out by comparing the peak relative retention times of the sample FAMEs with those obtained for Alltech standarts (Lexington, USA).

Statistical analyses: All analytical determinations and GC analyses were performed in triplicate and the mean values were reported. The statistical analyses of total lipid contents and percentages of fatty acid were tested by analysis of variance (ANOVA) and comparisons between means were performed with Tukey's test. Differences between means were evaluated as significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

The percentages of total lipid and total fatty acid in investigated *Achillea wilhelmsii* seeds according to altitude are presented in Table 1. The total lipid percentages of seeds at different altitude were found to be between 2.73 and 4.27%. The lowest lipid level (2.73%) was 1900 m and the highest (4.27%) at 1650 m. There were no significant difference between total fatty acid percentage at 1080 m (2.27%) and 1650 m (2.60%).

Table 1. Total lipid and total fatty acid percentages of seed of *A. wilhelmsii*

Altitude	Total lipid % (Mean±S.E.)*	Total fatty acid % (Mean±S.E.)*
1000-1080 m	4.17±0.08 a[C]	2.27±0.01 b
1600-1650 m	4.27±0.02 a	2.60±0.04 b
1850-1900 m	2.73±0.10 b	1.57±0.03 a

*Each value represents the mean of three experiments. [C]: (a, b) Values for each sample with different superscript letters in the same column are significantly different at $P \leq 0.05$.

Table 2. Comparison of fatty acid compositions (%) with altitude in *A. wilhelmsii* seed[A]

Fatty acids	1000-1080 m (Mean±S.E.)	1600-1650 m (Mean±S.E.)	1850-1900 m (Mean±S.E.)
C10:0[B]	0.26±0.00 a[C]	0.15±0.00 b	0.63±0.03 c
C12:0	0.47±0.01 a	0.32±0.00 a	0.68±0.01 b
C14:0	2.16±0.02 a	1.18±0.01 b	2.46±0.11 a
C15:0	0.15±0.00 a	0.11±0.00 a	0.23±0.00 b
C16:0	10.56±0.06 a	11.79±0.01 b	14.74±0.20 c
C17:0	1.06±0.45 a	0.24±0.01 b	0.79±0.04 a
C18:0	3.09±0.02 a	2.70±0.00 b	3.20±0.00 c
C20:0	2.32±0.02 a	2.11±0.01 a	2.47±0.02 b
C21:0	0.10±0.02 a	0.07±0.00 a	0.14±0.01 a
C22:0	1.75±0.03 a	1.55±0.01 b	1.85±0.03 b
∑SFA	21.92±0.06 a	20.23±0.01 b	27.19±0.06 c
C14:1	0.02±0.00 a	0.02±0.00 a	0.10±0.01 b
C15:1	0.25±0.01 a	0.12±0.00 c	0.19±0.01 b
C16:1	0.20±0.01 a	0.18±0.01 a	0.45±0.01 b
C17:1	0.02±0.00 a	0.02±0.00 a	0.06±0.01 b
C18:1 ω-9	10.46±0.02 a	17.23±0.02 c	12.94±0.01 b
C18:1 ω-7	0.65±0.00 a	0.70±0.00 b	0.70±0.04 b
C20:1 ω-9	0.27±0.01 a	0.46±0.02 b	0.66±0.01 c
∑MUFA	11.86±0.01 a	18.73±0.01 b	15.11±0.01 c
C18:2 ω-6	64.99±0.37 a	57.67±0.02 b	51.98±0.01 c
C18:3 ω-6(γ)	0.25±0.00 b	0.08±0.01 b	0.26±0.04 a
C20:2 ω-6	0.03±0.01 a	0.05±0.01 a	0.11±0.02 b
∑PUFA ω-6	65.27±0.13 a	57.80±0.07 b	52.35±0.02 c
C18:3 ω-3(α)	0.97±0.00 a	3.24±0.07 b	5.49±0.03 c
∑PUFA ω-3	0.97±0.00 a	3.24±0.07 b	5.49±0.03 c
∑UFA/SFA	3.52±0.00 a	3.78±0.01 a	2.48±0.01 b
ω-6/ω-3	67.28±0.03 a	17.84±0.01 b	9.53±0.01 c

[A]: Average of three lots analyzed. [B]: Values reported are means ± S.E. [C]: (a, b, c) Values for each sample with different superscript letters in the same row are significantly different at $P \leq 0.05$.
Σ SFA: total saturated fatty acid; Σ MUFA: total monounsaturated fatty acid; Σ PUFA ω-6: total ω-6 polyunsaturated fatty acid; Σ PUFA ω-3: total ω-3 polyunsaturated fatty acid.

But the total fatty acid percentage at 1900 m was the lowest (1.57%) and significant as statistically. The fatty acid compositions of the seed oils at different altitude and in the region are given Table 2. Twenty one fatty acids were identified. The fatty acid profiles in present study confirm the earlier reports with other *Achillea* species (Palic *et al.,* 2000; Goli *et al.,* 2008). Total saturated (∑SFA), monounsaturated (∑MUFA) and polyunsaturated (∑PUFA) acid contents of seed oils at different altitude were significantly different. But fatty acid profiles were quite similar. There were variations in the levels of some fatty acids between different altitude. Palmitic acid (C16:0) was predominat saturated fatty acid in all samples. This fatty acid amount at 1900 m was higher than other altitude (at 1080 and 1650 m). Myristic acid (C14:0), C16:0 and stearic acid (C18: 0), arachidic acid (C20:0) and behenic acid (C22:0) percentages have increased at 1900 m. These fatty acids have decreased at 1650 m. Capric (C10:0), lauric (C12:0), pentadecanoic (C15:0) and heneicosanoic (C21:0) acids also showed similar changes. It has been reported that C16:0 and C18:0 were the dominant SFAs in plant species as in our study (Akpınar *et al.,* 2001; Beyhan *et al.,* 2011). Ayaz *et al.,* (2016) emphasized that percentages of C16:0 and C18:0 were 17.69 and 12.59%, respectively. In addition, other SFAs contents such as C12:0, C14:0, C15:0, C22:0 and C24:0 were found averaged between 0.37-1.77%. In our study, significant difference was determined between ∑SFA percentages. ∑SFA of *A. wilhelmsii* at 1900 m was the highest level (27.19%). ∑SFA at 1080 m and 1650 m was low compared to 1900 m (21.92 and 20.23%, respectively). The major MUFA in the seed oils of A. wilhelmsii was oleic acid (C18:1 ω-9). (C18:1 ω-9) content was found higher level at 1650 m than at 1080 m and 1900 m. Myristoleic acid (C14:1), plamitoleic acid (C16:1), heptadecenoic acid (C17:1), vaccenic acid (C18:1 ω-7) and eicosenoic acid (C20:1 ω-9) have been found in low percentages (to be below 1 %). Also these fatty acid contents increased with altitude statistically significant (Table 2).

The ∑MUFA percentages did show very significant difference between investigated altitude. ∑MUFA content of seed oil of *A. wilhelmsii* at 1650 was higher (18.73%) than other altitude (11.86% at 1080 and 15.11% at 1900 m). However, the content of ∑MUFA decreased at 1080 m as statistical significant in seed oil. Essential fatty acids affect the fluidity, flexibility and permeability of membranes. At the same time, they are the precursors of eicosanoids. In general, lipids in plant contain excess amounts of ω-6 PUFA rather than ω-3 PUFA (Simopoulos 2016). Linoleic acid (C18:2 ω-6) was found in the greatest percentage in seed oil of *A. wilhelmsii*. This acid content was at the highest level at 1080 m sample (64.99%) but found to be at the lowest level at 1650 m (57.67 %) and 1900m (51.98%) samples. According to these results, C18:2 ω-6 percentages at 1900 m and 1650 m samples decreased with altitude as statistical. Also, Beyhan *et al.,* (2011) found that the concentrations of C16:0, C18:0, C18:2 ω-6 and C18:3 ω-3 decreased or unchanged in some *Coryllus avellama* varieties with altitude. In our study, γ-linolenic acid (γ-C18:3 ω-6) and eicosadienoic acid (C20:2 ω-6) contents were very low. At 1080 and 1900 m, γ-C18:3 ω-6 content did not change, but at 1650 m decreased with altitude. But, C20:2 ω-6 content increased at 1900 m sample. The percentage of ∑PUFA ω-6 because of the change in ω-6 series fatty acids was found to decrease with altitude as statistical. Any comparison could not be made because there were no studies on ω-6 and ω-3 series fatty acids of *Achillea* species. While the highest level (65.27%) of ∑PUFA ω-6 at 1080 m decreased to the lowest level (52.35%) at 1900 m and 1650 m (57.80%). C18:2 ω-6 content of seed oil of *A. wilhelmsii* was very richer than α-linolenic acid (α-C18:3 ω-3) content. Percentage of α-C18:3 ω-3 increased from 0.97% at 1080 m to 3.24% at 1650 m and 5.49% at 1900 m, unlike C18:2 ω-6 as statistical significant. In this study, ω-6/ω-3 ratios were 67.28% at 1080 m, 17.84% at 1650 m and 9.53% at 1900 m in seed oils *A. wilhelmsii.* Simopoulos (2016) recommend that because a high ω-6/ω-3 ratio is associated with overweight/obesity, where as a balanced ratio decreases obesity and weight gain, it is essential that every effort is made to decrease the ω-6 fatty acids in the diet, while increasing the ω-3 fatty acid intake. The reported that the concentrations of C18:2 and C18:3 in mature achene of *A. biebersteinii*, *A. bisserata*, *A. multifida* and *A. wilhelmsii* varied between 32.40-64.43% and between 2.72-16.45%, respectively (Ayaz *et al.,* 2016). Also, it has been established by many studies that fatty acid compositions of plant oils have been influenced by maturation stage of seed and fruit (Koyuncu *et al.,* 1997a, b), with temperature and regions within year (Pritchard *et al.,* 2000; Parcerisa *et al.,* 1993). However, our results demonstrated that it is understood that effects on fatty acid contents of *A. wilhelmsii* seed oil in differential altitude. Therefore, this study is first report about effect of altitude on fatty acid composition of *A. wilhelmsii* seed oil from Turkey flora.

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