EJONS International Journal on Mathematic, Engineering and Natural Sciences

ISSN 2602 - 4136

Article Arrival Date 15.05.2020 | Article Type Research Article | Article Published Date 12.06.2020

Doi Number: http://dx.doi.org/10.38063/ejons.245

DETERMINATION AND COMPARISON OF FATTY ACIDS OF *HELICHRYSUM ARENARIUM* PLANT IN TWO DIFFERENT REGIONS

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ABSTRACT

In this study, the fatty acid composition of the harvested *Helichrysum arenarium* plant at two different regions in Turkey was determined and compared by using GC-FID. Although the fatty acid levels of *Helichrysum arenarium* plant belonging to Nemrut Crater Lake around Bitlis Province in Eastern Anatolia region were determined as ΣPUFA 12.45 %, ΣMUFA 27.12 %, ΣUSFA 39.56 %, and ΣSFA 60.26 %, fatty acid levels of *Helichrysum arenarium* plant belonging to the mountainous areas of Sebinkarahisar district of Giresun Province in the Black Sea region were determined as ΣPUFA 32.21 %, ΣMUFA, 14.75 %, ΣUSFA 46.97 % and ΣSFA 52.69 %. The basic fatty acid content of *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake; 23.85 % Palmiteloic acid (16:1), 20.28 % Palmitic acid (16:0), 10.12 % Stearic acid (18:0), 6.50 % Heptadecanoic acid (17:0) and 6.01 % Linolenic acid (18:2n6c) were detected. The basic fatty acid content of *Helichrysum arenarium* plant belonging to the mountainous areas of Şebinkarahisar district of Giresun; 27.61 % Cis-4,7,10,13,16,19-docosahexaenoic acid (22:6n3), 13.03 % Palmitic acid (16:0), 12.53 % Palmiteloic acid (16:1), 8.84 % Stearic acid (18:1), 7.93 % Heptadecanoic acid (17:0) and 6.55 % Myristic acid (14:0) were detected. The ΣMUFA and ΣSFA fatty acid levels of the *Helichrysum arenarium* plant in the Eastern Anatolia region were observed that were higher than the Black Sea region. The ΣPUFA and ΣUSFA fatty acid levels of the Helichrysum arenarium plant in the Black Sea region were observed that were higher than the Eastern Anatolia region.

Keywords: *Helichrysum arenarium*, Fatty acid, GC-FID

1. INTRODUCTION

Helichrysum arenarium plant belongs to Asteraceae family and is a perennial herbaceous plant. People among known as golden grass, eternal flower, immortal flower or colorfast flower (Czinner, Lemberkovics, Bihátsi-Karsai, Vitányi, & Lelik, 2000; Albayrak, Aksoy, Sağdiç, & Budak, 2010; Eroğlu, Hamzaoğlu, Budak, Aksoy, & Albayrak, 2010; Moghadam, Sani, & Sangatash, 2014; Figas, Tomaszewska-Sowa, Sawilska, & Keutgen, 2016; Liu, Jing, & Li, 2019). The name *Helichrysum* derives in the Greek from “helios” which means the sun, and “chrysos” which means gold (Liu vd., 2019).

There are over 600 species of *Helichrysum* in the world. It is grow on sandy and semi-hard soils in the steppes of a wide geographical region including America, Scandinavia, Atlantic, Europe, Balkans, Russia, Siberia, the Caucasus, Asia Minor, Central Asia, Mongolia and China. It is also a perennial plant that is 90 cm high (Jarzycka, Lewińska, Gancarz, & Wilk, 2013; Reidel, Cioni, Ruffoni, Cervelli, & Pistelli, 2017; Kutluk, Aslan, Orhan, & Özçelik, 2018). *Helichrysum* species is 15 endemic in Turkey flora. It is represented by 27 taxa which are commonly found in Anatolia (Albayrak vd., 2010).

According to some pharmacological data *Helichrysum arenarium* flower is rich in phenolic compounds, including flavonoids, essential oils, fatty acids, carotenoids, steroids, bitter substances, polyphenols, vitamins, mineral salts, polysaccharides, glycosides, coumarins, catechinins and proanthocyanidins. In addition, it contains components such as astragaline, luteoline, kaempferol etc. (Rančić vd., 2005; Eroğlu vd., 2010; Jarzycka vd., 2013; Liu vd., 2019).

The flower of *Helichrysum arenarium* has many biological activities, including in particular antibacterial, antiviral, antifungal, antiinflammatory, antiallergic, antioxidant, antiproliferative, antimicrobial, antiradical, cholinergic, hepatoprotective and detoxification activities (Tepe, Sokmen, Askin Akpulat, & Sokmen, 2005; Mao vd., 2017; Liu vd., 2019). In folk medicine, it has been used to treat various ailments such as liver and gallbladder disorders, lumbago treatment, stomach pain, asthma, arthritis disorders, cystitis and jaundice treatment, skin infections, respiratory and digestive system disorders, kidney stones treatment, uro-genital disorders. It has also been used for many years for the smell in the cosmetic industry (Liu vd., 2019). It is generally used as herbal tea in Anatolia in our country (Eroğlu vd., 2010). It is used in South Africa to treat tuberculosis and related symptoms, and traditionally in Central Europe as antiseptic and spasmolytic drugs (Gradinaru, Silion, Trifan, Miron, & Aprotosoaie, 2014; Moghadam vd., 2014;Reidel vd., 2017; Akin & Saki, 2019).

In Turkey, no information is available in the literature about the fatty acid compositions of *Helichrysum arenarium*. This study was carried out to contribute to the literature. In this study, fatty acid compositions of the *Helichrysum arenarium* plant in the mountainous areas of Sebinkarahisar District of Giresun province in the Black Sea region and around Nemrut Crater Lake in Bitlis province in Eastern Anatolia region were determined by Gas Chromatography (GC-FID). In addition, Fatty acid compositions of *Helichrysum arenarium* plant belonging to two different regions were compared.

2. MATERIAL AND METHODS

2.1. Preparation of Plant Samples

The plant samples of *Helichrysum arenarium* in two different regions to be used in the study were collected from the Nemrut Crater Lake of the province of Bitlis in the Eastern Anatolia region and from the mountainous areas of Sebinkarahisar District of Giresun province in the Black Sea Region. Later, the collected plant samples were dried in a place with shadowy and air flow. The obtained samples were placed in a 10 mL capped vial and used in experimental studies.

2.2. Lipid Extraction of Plant Samples

1 g from *Helichrysum arenarium* plant belonging to two different regions was weighed and 15 mL of chloroform:methanol mixture was added over the plant at a volume ratio of 2:1 (v/v). The mixture was homogenized at a high speed homogenizer for 60 seconds. Later, the mixture was filtered through blue band filter paper and obtained the liquid mixture was used for methyl esterification.

2.3. Preparation of Methyl Esters of Plant Samples

The liquid mixture was transferred to a two-necked reaction flask. Over the mixture 3 mL methanol and 5 drops of sulfuric acid were added dropwise and heated at 85 °C for 2 hours with stirring in a reflux system. At the end of this process it was converted to methyl esters of fatty acids. Later, the mixture in the reaction flask was cooled and placed in a 50 mL capped tube. Hexane was added to the mixture and was mixed vortex for 30 seconds. So that methyl esters were extracted. Later, the mixture was taken upper phase and the fatty acid methyl esters were analyzed by GC-FID.

2.4. GC-FID Analysis of Plant Samples

Fatty acids of *Helichrysum arenarium* plant belonging to two different regions were converted to methyl esters. The sample was analyzed using SHIMADZU QP2020 gas chromatography (GC) instrument with FID detector. The RESTEK RTX-2330 capillary column (60 m x 0.25 mm x 0.20 µm) was used for analysis and helium as carrier gas. During the analysis, the injection and detector temperature was 250 °C and the column temperature was adjusted to 140-240 °C. Later, the column temperature was maintained at 140 °C for 5 min. and then, it was set to 240 °C at a 4 °C increase in min. Later, it was waited at 240 °C for 12 min. Analysis time was terminated as 42 min. The fatty acid methyl esters mixture of *Helichrysum arenarium* plant belonging to two different regions was injected into GC and the sample fatty acid analysis was performed.

3. RESULTS AND DISCUSSION

The two selected geographic regions have been chosen due to climatic differences, the structural differences of their geographical regions (Altitude, Geological structure of the region, etc.).

When the Table 1 was examined, the fatty acid levels of *Helichrysum arenarium* plant belonging to Nemrut Crater Lake in Bitlis province in Eastern Anatolia region were determined as ΣPUFA 12.45 %, ΣMUFA 27.12 %, ΣUSFA 39.56 % and ΣSFA 60.26 %. Fatty acid levels of *Helichrysum arenarium* plant belonging to the mountainous areas of Sebinkarahisar district of Giresun province in Black Sea Region were determined as ΣPUFA 32.21 %, ΣMUFA 14.75 %, ΣUSFA 46.97 % and ΣSFA 52.69 %. Total monounsaturated fatty acid (ΣMUFA) levels and total saturated fatty acid (ΣSFA) levels of *Helichrysum arenarium* plant in Eastern Anatolia region determined as higher than *Helichrysum arenarium* plant in Black Sea Region. Total polyunsaturated fatty acid (ΣPUFA) levels and total unsaturated fatty acids (ΣUSFA) levels of *Helichrysum arenarium* plant in Black Sea Region determined as higher than *Helichrysum arenarium* plant in Eastern Anatolia region. We think that reason that has different fatty acid levels of the *Helichrysum arenarium* plants in two different regions is due to the climatic and geographical region differences of the two regions. Kutluk et al., in a study did in 2018 are stated that the variability of plants in their chemical composition is related to genetic, geographical, and climatic factors (Kutluk vd., 2018).

Table 1. Percentage levels of total polyunsaturated fatty acids (ΣPUFA), total monounsaturated fatty acids (ΣMUFA), total unsaturated fatty acids (ΣUSFA) and total saturated fatty acids (ΣSFA) % levels of *Helichrysum arenarium* plant belonging to two different regions

Fatty acids	Name of Fatty Acid	Around B. N. K. G. belonging to *Helichrysum arenarium* fatty acid level (%)	G. S. M. A. belonging to *Helichrysum arenarium* fatty acid level (%)
18:2n6t	Linolelaidic acid	5.69±0.03	1.90±0.05
18:2n6c	Linolenic acid	6.01±0.01	2.69±0.11
20:3n6	Cis-8,11,14-eicosatrienoic acid	0.74±0.08	-
22:6n3	Cis-4,7,10,13,16,19-docosahexaenoic acid	-	27.61±0.04
ΣPUFA		**12.45±0.12**	**32.21±0.20**
15:1	Cis-10-pentadecanoic acid	3.27±0.07	-
16:1	Palmiteloic acid	23.85±0.02	12.53±0.09
18:1n9c	Oleic acid	-	2.22±0.07
ΣMUFA		**27.12±0.09**	**14.75±0.16**
ΣUSFA		**39.56±0.21**	**46.97±0.36**
10:0	Capric acid	-	1.78±0.02
12:0	Lauric acid	2.99±0.11	-
14:0	Myristic acid	5.16±0.14	6.55±0.09
15:0	Pentadecanoic acid	-	5.88±0.03
16:0	Palmitic acid	20.28±0.01	13.03±0.02
17:0	Heptadecanoic acid	6.50±0.06	7.93±0.11
18:0	Stearic acid	10.12±0.02	8.84±0.01
21:0	Heneicosanoic acid	5.43±0.04	4.16±0.07
22:0	Behenic acid	3.85±0.05	0.39±0.13
23:0	Tricosanoic acid	5.90±0.07	4.11±0.03
ΣSFA		**60.26±0.50**	**52.69±0.51**

B. N. K. G: belongs to Bitlis Nemrut Crater Lake

G. S. M. A: belongs to the Mountainous Areas of Sebinkarahisar district of Giresun

The basic fatty acid content of *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake; as 23.85 % Palmiteloic acid (16:1), 20.28 % Palmitic acid (16:0), 10.12 % Stearic acid (18:0), 6.50 % Heptadecanoic acid (17:0) and 6.01 % Linolenic acid (18:2n6c) was determined.

The basic fatty acid content of *Helichrysum arenarium* plant belonging to the mountainous areas of Sebinkarahisar district of Giresun; as 27.61 % Cis-4,7,10,13,16,19 docosahexaenoic acid (22:6n3), 13.03 % Palmitic acid (16:0), 12.53 % Palmiteloic acid (16:1), 8.84 % Stearic acid (18:1), 7.93 % Heptadecanoic acid (17:0) and 6.55 % Myristic acid (14:0) was determined.

In Table 1, Although the highest fatty acid content of the *Helichrysum arenarium* plant belonging to around Bitlis Nemrut Crater Lake is Palmiteloic acid (16:1), the highest fatty acid content of *Helichrysum arenarium* plant belonging to the mountainous areas of Sebinkarahisar district of Giresun is Cis-4,7,10,13,16,19-docosahexaenoic acid.

When Table 1 is examined, it is seen that Palmiteloic acid (16:1), Palmitic acid (16:0), Stearic acid (18:0) and Heptadecanoic acid (17:0) levels are high in both plant species.

When Table 1 is looked, levels of Palmiteloic acid (16:1), Palmitic acid (16:0) and Stearic acid (18:0) of *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake are seen higher than *Helichrysum arenarium* plant belonging to the mountainous areas of Sebinkarahisar district of Giresun.

When Table 1 is examined, it is seen that the Linolenic acid (18:2n6c) level of *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake is higher than *Helichrysum arenarium* plant belonging to the mountainous areas of Sebinkarahisar district of Giresun.

Palmitic acid (16:0) and Stearic acid (18:0) are saturated fatty acids and one of the most important fatty acids found in vegetable oils. In particular, Stearic acid (18:0) and Palmitic acid (16:0) are the most common saturated fatty acids and are synthesized from the human body (Karaca & Aytaç, 2007).

Linoleic acid (18:2n6c), which is an essential omega-6, is necessary for the transmission of cell membranes of the human body, brain function and nerve impulses under normal conditions. Furthermore, these essential fatty acids play role in atmospheric oxygen transfer to the blood, hemoglobin synthesis and cell division (Lopes, Bruna R. Böger, Kelen F. Cavalli, Silveira-Júnior Osório, Daniel V. C. L., Débora F. de Oliveira, & and Tonial, 2014).

Docosahexaenoic acid (22:6n3) is an endogenous compound that is important for the retina, brain and sperm. It is also a crucial fatty acid for brain development as well as learning ability and visual acuity. In terms of health, Docosahexaenoic acid (22:6n3) is an important compound used in the prevention of cardiovascular diseases (Çakmakçı & Tahmas-Kahyaoğlu, 2012).

4. CONCLUSION

ΣPUFA, ΣMUFA, ΣUSFA and ΣSFA fatty acid levels of *Helichrysum arenarium* plants in two different regions were examined. ΣMUFA and ΣSFA fatty acid levels of *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake were observed that is higher than *Helichrysum arenarium* plant belonging to the mountainous areas of Şebinkarahisar district of Giresun. ΣPUFA and ΣUSFA fatty acid levels of *Helichrysum arenarium* plant belonging to the mountainous areas of Şebinkarahisar district of Giresun were observed that is higher than *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake.

Since PUFA are known to be beneficial for health, PUFA content of the fatty acids of *Helichrysum arenarium* plant belonging to mountainous areas of Şebinkarahisar district of Giresun is higher than *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake. Therefore, we think that *Helichrysum arenarium* plant, which belongs to the mountainous areas of Şebinkarahisar district of Giresun, is beneficial for human health as herbal tea.

Linolenic acid (18:2n6c) level of *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake is higher than *Helichrysum arenarium* plant belonging to the mountainous areas of Şebinkarahisar district of Giresun. Due to the importance of Linolenic acid mentioned above, it shows that *Helichrysum arenarium* plant around Bitlis Nemrut Crater Lake can be used as a drug active substance.

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