RESEARCH ARTICLE / *Araştırma Makalesi*

Analysis of the fatty acids from *Ferula elaeochytris* root extract by GC/FID

GC/FID ile Ekstrakte Edilen *Ferula elaeochytris* Kök Ekstresinden Yağ Asidlerinin Analizi

Nadire Eser[1], Atila Yoldas[2], Feridun Kocer[3]

[1] Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Pharmacology, Kahramanmaras
[2] Kahramanmaras Sutcu Imam University, Faculty of Medicine, Department of Anatomy, Kahramanmaras
[3] Kahramanmaras Sutcu Imam University, Research and Development Centre for University-Industry-Public Relations, Kahramanmaras

Yazışma Adresi / *Correspondence:*
Nadire Eser

Kahramanmaraş Sütçü İmam Üniversitesi, Tıp Fakültesi, Tıbbi Farmakoloji Anabilim Dalı, 46100, Kahramanmaras, Türkiye
T: **+90 544 657 42 21** E-mail **: esernadire01@hotmail.com**

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Orcid :
Nadire Eser; https://orcid.org/0000-0003-1607-5114
Atila Yoldas; https://orcid.org/0000-0002-7807-0661
Feridun Kocer; https://orcid.org/0000-0002-8749-7106

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Abstract

Objective	*Ferula elaeochytris* root (FE) which is commonly used by local people to treat many diseases and disorders, has an aromatic fragrance, because of its oils and oleoresin content. We aimed to identificate the fatty acid components of *Ferula elaeochytris* root extract and importance for human body.
Materials and Methods	In the present study, Fatty acid profile was analysed of the *Ferula elaeochytris* root extract, collected from Engizek plateau of Kahramanmaraş and prepared by withering and triturating, by using a Gas Chromatograph equipped with Flame Ionization Detector (GC-FID).
Results	A total of 16 fatty acids of which nine are unsaturated and seven are saturated were determined. Unsaturated (56.25%) fat content was higher than saturated fat (43.75%). Omega-6-derived linoleic acid (39,25%) as an unsaturated fatty acid that needs for to be dietary supplied to human body and omega-3 derived alpha-linolenic acid (2,390%) contents were found to be high whereas omega-9 derived oleic acid (15.79) was the second most abundant. The medicinal herb also contained Nervonic acid (0.599%) which is important for development and disorders of nervous system. The palmatic acid (10,506%) was found to be the highest levels among unsaturated fatty acids.
Conclusion	In this study, major components in *Ferula elaeochytris* content, which have shown many beneficial effects on human health, have been demonstrated by analysis with GC-FID.
Keywords	Fatty acids, chromatography, antioxidants, Gas Chromatography/Flame Ionization Detector

Öz

Amaç	*Birçok hastalığın tedavisinde yerel halk tarafından yaygın olarak kullanılan Ferula elaeochytris kökü (FE), yağları ve oleoresinleri içermesi yönüyle güçlü bir aromatik kokuya sahiptir. Bu çalışmada, Ferula elaeochytris kök ekstresinin yağ asidi bileşenlerini ve insan vücudu için önemini belirlemek amaçlandı.*
Gereç ve Yöntemler	*Bu çalışmada, Kahramanmaraş'ın Engizek platosundan toplanan Ferula elaeochytris bitkisinin kök kısmı kurutulup, öğütülerek hazırlanan kök ekstresinin yağ asidi profili, Alev İyonizasyon Dedektörleri (GC-FID) ile donatılmış bir Gaz Kromatografisi kullanılarak analiz edildi.*
Bulgular	*Yapılan analiz sonucunda 9 adet doymamış, 7 adet doymuş toplam 16 yağ asidi belirlenmiştir. Doymamış (% 56,25) yağ içeriği, doymuş yağdan (% 43,75) daha yüksek bulundu. insan vücudunun besin ihtiyacının sağlanması için gereken doymamış yağ asidi olarak Omega-6'dan türetilmiş linoleik asit (% 39,25) ve omega-3 türevi alfa-linolenik asit (% 2,390) içeriği yüksek iken, omega-9 türevi oleik asit (15.79) en bol bulunan ikinci sıra yağ asidi olarak bulunmuştur. Bu bitki ayrıca sinir sisteminin gelişimi ve bozuklukları için önemli olan Nervonik asit (% 0.599) içermektedir. Palmatik asit (% 10,506), doymamış yağ asitleri arasında en yüksek seviyelerde bulundu.*
Sonuç	*Yağ asitleri, kromotografi, antioksidanlar, Gaz Kromatografisi/Alev İyonizasyon Dedektörü*

INTRODUCTION

For centuries, medicinal herbs have emerged as important medicinal sources and currently constitute about 25% of pharmaceutical prescriptions.[1,2] According to data from the world health organization (WHO), more than 80% of the population in the third world countries depend on traditional medical methods and the herbs will continue to play a vital role in the health system. Due to the rapidly deteriorating ecological balance, many types of current medicinal plant resources or species that have potential to be used as medicinal plants face extinction. It is also known that transformation in the soil structure can change the components in a given plant species even if does not drive to the extinction.[1] Therefore, it is important to discover the components found in plants and to identify active principles in order to scientifically verify the traditional medicinal plants and subsequently use them as therapeutic drugs. It is emphasized that in addition to the effects of the main constituents, the secondary plant metabolites are also important for human health, and thus revealing the main components of plants is one of the most important steps of phytochemical research.[2]

About 170 different species of Ferula have been identified. In pharmacological researches carried out in some species, in addition to the aphrodisiac, antioxidant, anti-inflammatory, antimicrobial, antifungal, antispasmodic, antiulcerative and hepatoprotective effects of the plant, the plant extracts in experimental studies indicated that it might have positive contribution to the erectile function and fertility and reduce diabetes and infertility.[3-8] *Ferula elaeochytris* Korovin is a species that grows in Eastern Mediterranean regions such as Syria, Lebanon, Kahramanmaraş and Hatay provinces of Turkey. In the analyzes conducted on Ferula elaeochytris collected in Hatay, Kahramanmaraş and Bayburt regions, it was found that plants either contained different components or same components with varying quantities and some of these components had important antioxidative and anti-inflammatory effects.[9-11] In addition, experimental studies have revealed that it has positive results on erectile dysfunction due to, demonstrated cytotoxic activities on the human chronic myeloid leukemia and mouse leukemia cell line, produced antimicrobial effects, and was important in the rumen fermentation.[12-15]

Oil components of the medicinal plant origin contain many valuable structural elements and also being used as an important energy source.[16] In recent years, increased research on fatty acids has been conducted and positive results have been obtained on diseases such as cancer, MS, Alzheimer's disease and aging, as well as its sedative and hypnotic effects.[17-23] Therefore, the aim of this study was to investigate the fatty acid composition of the root extract of Ferula elaeochytris which has been used for centuries and to shed a light for further studies by revealing the known biological effects.

MATERIALS and METHODS

Plant material

Roots of *F. elaeochytris* were collected from the Engizek plateau (Kahramanmaras) in June 2017. The specimens were authenticated by Dr. Mustafa Aslan (a taxonomist). A voucher specimen (Voucher number HURUB 4588-4589) has been kept in the herbarium of the Department of Biology Education, Faculty of Education, Harran University, Sanlıurfa, Turkey.

Sample preparation

Soil and foreign materials on the outer surface of the *F. elaeochytris* roots were removed, and dried on a clean, dry surface to ensure no decomposition occurred. An amount of 0.1 g oil extract was placed in 15 ml cap tube and 1 ml 2 N Methanol KOH solution was added, vortexed for 2 min and put in rest for 15 min. Then 10 ml of hexane was added to the mixture, centrifuged at 7000 rpm for 10 minutes to obtain a phase separation. A total of 1 µl of the supernatant was drawn for GC injection and inserted to the GC vial.

Extraction methods of *Ferula elaeochytris*

Soxhlet device was used for oil extraction from the root of Ferula elaeochytris. For this purpose, grinded 20 g *Ferula elaeochytris* root was loaded into the cartridge and 250 mL of hexane was added and extraction was proceeded for 4 hours at 60 °C.[24] The hexane was then removed in the evaporator. The extract was stored at +4 °C until use.

Fatty acid composition with GC-FID

After the fatty acids were methylated, they were analyzed Shimadzu Gas Chromatography (Model 2025) with the Flame Ionization Detector (FID). The certified standard Supelco 37 Component Mix from Supelco Company was used in the analysis process. The standard mixture of 37 was used in the fatty acid methyl ester analysis. The mixture contains trans-cis, omega-3 and omega-6 fatty acids. Teknocroma brand TR-CN100 column was used in the study. The length was 60 m, the film thickness was 0.25 micron and the inner diameter was 0.20mm. For the heating of the column, it was initially kept at 80 °C for 2 minutes. Then, an incremental increase of to 5 °C per minute was applied and kept at the temperature of 140 °C for 2 minutes. This was followed by a further incremental increase of 3 °C per minute until it reached to the 240 °C and kept there for 5 min. Total analysis time was 61 minutes. The injector temperature was 240 °C and the detector temperature was 250 °C. Helium was used as the carrier gas and the flow rate was set to 30 ml/min. The gas flows used were determined as H2=40ml/min and dry air=400 ml/min.

Statistical analysis

Data were analysed with the SPSS-23 statistical software. The data are reported as the mean of triplicate measurements.

RESULTS

The 16 fatty acid profiles in the *Ferula elaeochytris* root extract with GC-FID analysis are shown in Fig. 1. A total of 16 (100%) fatty acids detected in the Ferula root extract composition nine (56.25%) of which were unsaturated fatty acids (Table 1) and seven (43.75%) were saturated fatty acids (Table 2). Linoleic acid, Oleic acid (unsaturated), and Palmitic acid (saturated) were determined to be the major components. Linoleic acid is in omega-6 (ω6) and Oleic acid is in omega-9 (ω9) class (Table 1).

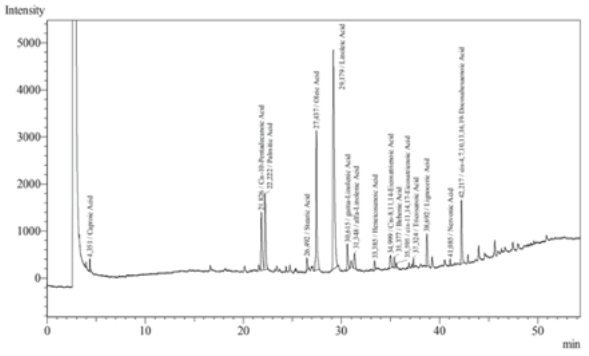

Fig. 1. GC-FID chromatogram of the Fatty acids of FE

Table 1. Unsaturated fatty acid Composition of *Ferula elaeochytris* root extract

No	RT (min)	Name of the compound	Concentration (%)	carbon number
1	29,179	Linoleic Acid	39,639±0,445	C 18:2 ω6
2	27,437	Oleic Acid	15,534±0,262	C 18:1 ω9
3	21,826	Cis-10-Pentadecanoic	8,407±0,043	C 15:1 ω5
4	42,217	Dokosahexaenoic acid	7,353±0,221	C 22:6 ω3
5	30,615	γ-linoleic acid	3,161±0,039	C 18:3 ω6
6	31,348	α-linoleic acid	2,268±0,132	C 18:3 ω3
7	34,999	cis-8-11-14-Eicosatrienoic Acid	2,237±0,355	C 20:3 ω6
8	41,085	Nervonic acid	0,556±0,087	C 24:1 ω9
9	35,595	cis-11,14,17-Eicosatrienoic Acid	0,519±0,085	C 20:3 ω3

Linoleic acid (39,242%) was determined to be the highest component and oleic acid (15,792%) was the second highest among major unsaturated fatty acids. It was also determined that the nervonic acid (0,599%) and cis-11,14,17-Eicosatrienoic acid (0,530%) were the minor components (Table 1).

Palmitic acid (10,506%) was the most abundant among the components of saturated fatty acid in the Ferula root ex-

tract followed by Lignoceric acid (4,438%) (Table 2). Palmitic acid and Lignoceric acid were found to be the major saturated fatty acid components in the Ferula root extract (Table 2).

Table 2. Saturated fatty acid Composition of *Ferula elaeochytris* root extract

No	RT (min)	Name of the compound	Concentration (%)	carbon number
1	22,222	Palmitic acid	10,524±0,294	C 16:0
2	38,692	Lignoceric Acid	4,449±0,237	C 24:0
3	26,492	Stearic Acid	1,317±0,169	C 18:0
4	35,377	Behenic Acid	1,343±0,083	C 22:0
5	33,385	Heneicosanoic Acid	1,143±0,077	C 21:0
6	4,351	Caproic Acid	0,835±0,077	C 6:0
7	37,324	Tricosanoic Acid	0,715±0,034	C 23:0

DISCUSSION

Fatty acids are divided into two main groups as saturated and unsaturated according to type of bond, they contain. It has been reported that saturated fatty acids can cause obesity with cardiovascular diseases and the effect of unsaturated fatty acids are generally positive.[25,26] However, it has also been reported in the previous studies that its intake is crucial for human health when it is consumed in certain amounts and in a balanced manner (Table 3). In the present study, unsaturated fatty acids were found to be more than saturated fatty acids. Palmitic acid was found to be highest among saturated fatty acids (Table 2). It has been reported that this fatty acid has antitumor effect in addition to the usage in many areas including cosmetic industry.[27]

In our study, unsaturated fatty acid compounds from ω-3, ω-6, and ω-9 groups that are reported to be important for human health were detected. Unsaturated fatty acids are classified into three groups as ω-3, ω-6, and ω-9. Linoleic acid (Omega 6) and alpha-linolenic acid (Omega 3) are the fatty acids that must be taken supplemented through the diet.[26] In the current study, the most abundant component of the Omega-6 group was found to be linoleic in Ferula root extract. Although it is controversial whether cancer-related results are positive or not, it is known that obesity and cardiovascular diseases are prevented by using anti-inflammatory agents of this fatty acid when consumed in a balanced manner.[28]

The presence of alpha-Linolenic acid (αLNA; 18: 3n-3), a major fatty acid in the human diet, was detected in our study. This acid is a longer chain, more unsaturated n-3 fatty acid that imparts significant biophysical properties in tissue function and cell membranes and is a substrate for the synthesis of EPA (20: 5n-3) and DHA (22: 6n-3).[29]

In our study, oleic acid, which is the precursor of Omega-9 group fatty acids from monounsaturated fatty acids, was second most abundant after linoleic acid. It was found in the structure of cell membrane and lipoproteins and protects these structures against oxidative stress.[30] Diets containing oleic acid and high levels of monounsaturated fatty acids are known to reduce the levels of triglycerides, cholesterol, decrease the blood pressure, and decrease the sensitivity of LDL cholesterol to oxidation.[31] In many in vitro and in vivo studies, the effect of oleic acid on cancer was investigated extensively and the reducing effect of the risk of developing breast, colorectal, lung and prostate cancer is reported.[32] In relation to diabetes, monounsaturated fatty acids are reported to improve glucose balance and insulin sensitivity, decrease plasma glucose and insulin concentration.[33]

The other fatty acid detected here is Docosahexaenoic acid. In the experimental study, it was determined that this oil strengthened memory and prevented Alzheimer.[34] Doxahexsaenoic acid (DHA) was reported to be essential for the maintenance of normal brain function in adults and for growth and functional development of the brain in infants. The inclusion of abundant DHA in the diet was reported to improve learning ability, while DHA deficiency leaded to learning difficulties.[25]

Nervonic acid detected in this study is the lowest unsaturated fatty acid component and have been the subject of fewer studies. Nervonic acid was reported to increase brain function and prevent demyelination. Studies have shown a negative correlation between nervonic acid and risk factors associated with obesity.[26] In addition, plasma nervonic acid is expressed as a potential biomarker for major depressive disorder.[35]

Table 3. Bioactivity of Saturated/Unsaturated fatty acids identified in the FE

Name of the compound	Major Biological Activities	Fatty Acids	Literatures
Palmitic acid	Cancer-Preventive	Saturated	Mancini et al.27
Lignoceric acid	Acid oxidation in skin fibroblasts	Saturated	Busnella et al.3[1]
Stearic Acid	Acid oxidation in skin fibroblasts	Saturated	Busnella et a[l.31]
Behenic Acid	Cholesterol-enhancing	Saturated	Cater and Denke[36]
Heneicosanoic Acid	Required for vitamin B12. Conversion of propionate to succinate, omegaoxidation	Saturated	Bralley[37,] Ernst[38]
Caproic Acid	In hemorrhagic syndrome	Saturated	Charytan and Purtilo[39]
Tricosanoic Acid	Required for vitamin B12. Conversion of propionate to succinate, omegaoxidation	Saturated	Bralley[37]
Linoleic Acid	Hypocholesterolemic, Anti-inflammatory,	Unsaturated	De Meester and Watson[40]
Oleic Acid	Hypocholesterolemic, Cancer-Preventive, Antiinflammatory	Unsaturated	De Meester and Watson[40]
Cis-10-Pentadecanoic	Associated with cancer	Unsaturated	Brown et al.[41]
Dokosahexaenoic acid	Anti-inflammatory, Hypocholesterolemic, In functions of cardiac and brain, anti-cancer	Unsaturated	De Meester and Watson[40,] Calder[42,] De Pablo[43]
γ-linoleic acid	Anti-inflammatory, anti-cancer, rheumatoid arthritis	Unsaturated	Innes and Calder[44]
α-linoleic acid	Anti-cancer, diet	Unsaturated	Burdge and Calder[29,] De Meester and Watson[40,] Calder[42]
cis-8-11-14- Eicosatrienoic Acid	Anti-inflammatory	Unsaturated	De Pablo[43]
Nervonic acid	In functions of nervous system	Unsaturated	Kageyama[35,] Derbyshire[45]
cis-11,14,17- Eicosatrienoic Acid	Anti-inflammatory, in cardiac functions, anti-cancer	Unsaturated	De Meester and Watson[40,] Calder[42,] De Pablo[43]

CONCLUSION

The analysis of *Ferula elaeochytris* root oil extract revealed that the amount of unsaturated fatty acid is greater than the amount of saturated fatty acid and those unsaturated fatty acids include the ones that prevent against human cardiovascular system and nervous system disorders, diabetes, chronic diseases and increase wound healing, antioxidative and anti-inflammatory effects. This justifies the use of this root extract for various ailments by the local populations. However, isolating phytochemical components from the plant and testing their pharmacological activity in vivo to determine the effects of both primary and secondary components would be invaluable.

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