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Research Article

Fatty Acid Composition Analysis of Some Apiaceae Plants Using Gas Chromatography-Flame Ionization Detector (GC-FID)

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ABSTRACT

Background/aim: The Apiaceae family, also referred to as the parsley or carrot family, comprises a diverse group of plants with significant ecological and economic importance. The fundamental building blocks of plant lipids, fatty acids are involved in a number of critical biological functions. Flame ionization detection in gas chromatography (GC-FID) is a potent analytical method widely used for the qualitative and quantitative analysis of fatty acid composition in plant samples.
Materials and methods: In this study, we investigated the fatty acid profiles of several Apiaceae plants using GC-FID to elucidate their lipid composition and potential applications.
Results: A wide spectrum of fatty acids was confirmed, ranging from C6:0 to C22:6. *Astrodaucus orientalis* leaf and fruit were found rich in palmitic acid (C16:0; 35.96% and 37.65%, respectively). *Ferulago asparagifolia* was determined as the richest sample in terms of poly-unsaturated fatty acids such as linoleic acid (C18:2 cis, 25.65%), alpha linoleic acid (C18:3n3, 7.42%) and eicosadienoic acid (C20:2, 32.66%). *Ferulago syriaca* ethanol and hexane extracts contained considerable amount of oleic acid (C 18:1 n9, 40. 37% and 49.19%, respectively).
Conclusion: Our review of the literature revealed that no prior reports have been made about the fatty acid compositions of *Astrodaucus orientalis, Ferulago asparagifolia and Ferulago syriaca*. As a result, the information provided here may be the first to describe the fatty acid contents of these species. Exploring the lipid profiles of these plants can enhance their potential applications in the food, pharmaceutical, and cosmetic industries.

Araştırma Makalesi

Gaz Kromatografisi-Alev İyonizasyon Dedektörü (GC-FID) Kullanılarak Bazı Apiaceae Bitkilerinin Yağ Asidi Kompozisyon Analizi

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ÖZ

Giriş/amaç: Genellikle havuç veya maydanoz ailesi olarak bilinen Apiaceae ailesi, önemli ekolojik ve ekonomik öneme sahip çeşitli bir bitki grubunu içerir. Yağ asitleri bitki lipitlerinin temel bileşenleridir ve çeşitli biyolojik süreçlerde önemli roller oynarlar. Gaz kromatografisi- alev iyonizasyon tespiti (GC-FID), bitki örneklerindeki yağ asidi bileşiminin kalitatif ve kantitatif analizi için yaygın olarak kullanılan güçlü bir analitik tekniktir.
Gereçler ve yöntemler: Bu çalışmada, lipid kompozisyonlarını ve potansiyel uygulamalarını aydınlatmak için GC-FID kullanarak çeşitli Apiaceae bitkilerinin yağ asidi profillerini araştırdık.
Sonuçlar: C6:0'dan C22:6'ya kadar değişen geniş bir yağ asidi spektrumu doğrulandı. *Astrodaucus orientalis* yaprak ve meyvesi palmitik asit (C16:0; sırasıyla %35.96 ve %37.65) bakımından zengin bulunmuştur. *Ferulago asparagifolia* linoleik asit (C18:2 cis, %25.65), alfa linoleik asit (C18:3n3, %7.42) ve eikosadienoik asit (C20:2, %32.66) gibi çoklu doymamış yağ asitleri açısından en zengin örnek olarak belirlenmiştir. *Ferulago syriaca* etanol ve hekzan ekstraktları önemli miktarda oleik asit (C 18:1 ω9, sırasıyla %40.37 ve %49.19) içermektedir.
Sonuç: Yaptığımız literatür araştırmasına göre, Astrodaucus orientalis, *Ferulago asparagifolia* ve *Ferulago syriaca'nın* yağ asidi bileşimleri daha önce rapor edilmemiştir. Bu nedenle, burada sunulan veriler yağ asidi bileşimlerine ilişkin ilk rapor olarak kabul edilebilir.

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Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur (N. Eruygur).	It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (N. Eruygur).
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1. Introduction

The Apiaceae family includes a large number of plant species distributed worldwide, many of which are utilized for their medicinal, culinary, and aromatic properties. Fatty acids are key constituents of plant lipids, such as triglycerides, phospholipids, and glycolipids, and they contribute to the nutritional value and sensory characteristics of plant-based products (Burdge and Calder, 2015). Moreover, fatty acids are also important in plant physiology, as they participate in membrane structure, energy storage, and signaling pathways. Specifically, the Apiaceae family is known for containing unique fatty acids such as petroselinic acid, which has notable biological activities and applications in food, pharmaceuticals, and cosmetics (Wang et al., 2022). This diversity and functionality of fatty acids in the Apiaceae family underscore their importance in both human nutrition and plant physiology (Hajib et al., 2023).

Members of the *Ferulago* W. Koch genus, belonging to the Apiaceae family, are perennial species. The genus comprises 34 taxa, 19 of which are endemic to Turkey, where they are commonly known by various names such as "çakşır, çağşır, kargı kişnişi, günlükotu, kılkuyruk, kuzukemirdi, şeytanteresi, kaya kişnişi, kır kişnişi" (Saya, 2012). These plants have traditional medicinal uses, including as carminatives, peptics, aphrodisiacs, vermifuges, and for treating ulcers, headaches, snake bites, hemorrhoids, and spleen diseases. They are also used in salads or as spices and are eaten by goats and deer (Erdurak, 2003). Certain *Ferulago* species' roots are used in Turkish traditional medicine for its aphrodisiac qualities as well as for the treatment of cancer and dermatological conditions, while the aerial parts are used as sedatives, immunostimulants, flavors, digestives, tonics, vermicidals, and anti-bronchitis agents (Bulut et al., 2014; Demirci et al., 2014; Karakaya et al., 2018). *Ferulago asparagifolia* Boiss. is a perennial herb which grows in the Western, Southern and Central Anatolia(Alkhatib et al., 2009). The glaucous, perennial, and glabrous species *F. syriaca* Boiss. is found in Hatay, Antalya, and South Anatolia (Peşmen, 1972). One of the other Apiaceae plant, known by most as "mountain carrot" *Astrodaucus orientalis* (L.) Drude is an herbaceous, erect, glabrous perennial plant with a nice scent. It is widely distributed across various regions particularly in fields, slopes, and along roadsides at altitudes ranging from 350 to 2700 meters. This aromatic plant is traditionally used in some parts of Iran and Turkey as a salad, vegetable, and food additives (Nazemiyeh et al., 2009).

Plant seeds are an excellent source of unsaturated fatty acids among other fatty acids. These seeds play a crucial role in providing essential nutrients and healthy fats that are beneficial for overall health (Kapoor et al., 2021). Fatty acids are carboxylic acids with carbon chains ranging from 2 to 36 carbon atoms. They can be classified as either saturated or unsaturated. Monounsaturated and polyunsaturated fatty acids are two types of unsaturated fatty acids. They are important for health because they protect against cardiovascular diseases neurological disease, autoimmune disorders, diabetes, arthritis and arrhythmia (Czumaj and Śledziński, 2020). Polyunsaturated fatty acids are a type of unsaturated fatty acid characterized by having multiple double bonds in a pentadiene configuration along their carbon chain (Tvrzicka et al., 2011). Plant seeds are excellent sources of alpha-linolenic acid (ALA), which is an omega-3 fatty acid. ALA is converted in the body to other beneficial omega-3s like eicosapentaenoic acid and docosahexaenoic acid. Omega-3 fatty acids are known for their anti-inflammatory properties and benefits for heart and brain health. Omega-6 fatty acids including linoleic acid (LA), are essential for the body and play a role in skin health, hormone production, and immune function (Damude and Kinney, 2008).

Gas chromatography-Flame Ionization Detector (GC-FID) is a well-established analytical technique for fatty acid analysis due to its high sensitivity, resolution, and ability to identify and quantify a wide range of compounds (Ruiz-Rodriguez et al., 2010). To the best of our knowledge, the fatty acid composition of *A. orientalis, F. asparagifolia* and *F. syriaca* have not been subject of previous study until now. By analyzing the fatty acid composition of some Apiaceae plants using GC-FID, we can gain insights into their lipid profiles, identify unique fatty acid constituents, and explore their potential applications in various industries.

2. Materials and Methods

2.1. Plant material

Plant materials from *A. orientalis*, *F. asparagifolia*, and *F. syriaca* were gathered and authenticated by Prof. Dr. Yavuz Bagci. Voucher specimens are stored in the herbarium of the Faculty of Science at Selcuk University (KNYA), Turkey. Details regarding collection and extract yield are provided in Table 1.

2.2. Extraction

The powdered plant materials were packed in Soxhlet extraction apparatus. Distillation was conducted with n-hexane and then with ethanol separately for each sample for about 4 h. The solvent was removed under reduced pressure using a rotary evaporator (Buchi R-100, Swiss). Recovered fixed oils were transferred in an amber glass bottle and stored at -20°C. The extracted fixed oils were then derivatized with their methyl esters and analyzed using GC-FID equipped with a capillary column for separation and mass spectrometric detection.

Table 1. The collecting information and extract yield of plant materials.

Plant Name	Herbarium /Voucher no.	Locality	Solvent	Yield (g g^{-1})
Astrodaucus orientalis (L.) *Drude*	Bağcı 4209	C4: Karaman-Çakırdağı: Yalnızdağ Hill, rocky slopes, around 1200 m, 14.07.2021	Hexane	Leaf: 2.38% Fruit: 11.20%
			Ethanol	Leaf: 17.82% Fruit: 2.92%
Ferulago asparagifolia Boiss	Bağcı 4206	C4: Antalya; between Gazipaşa-Alanya 10 km, ca. 50 m, 20.07.2021	Hexane	Leaf: 3.02% Fruit: 6.89%
			Ethanol	Leaf: 16.02% Fruit: 2.92%
Ferulago syriaca Boiss.	Bağcı 4205	C6: Hatay, Yayladağ road, rocky places, 450-480 m, 28.07.2021	Hexane	9.78%
			Ethanol	9.37%

2.3. Synthesis of fatty acid methyl esters

Fatty acid methyl esters (FAMEs) in plant extracts must be derivatized and converted into volatile form to be analyzed by gas chromatography. For this purpose, sample preparations were completed by considering the EU regulation (Burdge & Calder, 2015). An average of 0.1000 g of the plant extract was weighed and taken into the sample bottle. 10 mL of hexane was added to each extract and vortexed for 10 seconds. The samples were vortexed after the addition of 100 μL of 2 N potassium hydroxide solution in methanol. The solutions were shaken on a vortex for 1 minute. Samples were centrifuged at 2500 × g for 5 min to separate the phases. The supernatant was taken from the samples, whose phases were completely separated, into vials and injected into GC-FID.

2.4. FAME analysis by GC-FID

The analysis of FAME was conducted using the Agilent 6890N gas chromatography system equipped with an FID detector(Agilent Technologies Inc., Wilmington, DE, USA). Separation was performed with a high polarity HP-88 column designed for the separation of FAMEs. Helium gas was used as the mobile phase. The injection and detector temperatures were maintained at 250 °C. A gradient temperature program was employed as follows: starting at 45 °C for 0–4 minutes, increasing by 13 °C from 4 to 27 minutes to reach 175 °C, holding at 175 °C for 27 minutes, then increasing by 4 °C from 27 to 35 minutes to 215 °C, and maintaining at 215 °C for 35 minutes. Samples were injected using split injection mode (1 μL). The hydrogen flow rate was 30 mL/min and the dry air flow rate was 300 mL/min. Chromatograms were acquired using the Agilent 1200 Series-B.03.02 software.

3. Results and Discussion

Analysis using GC-FID identified a variety of fatty acids present in the Apiaceae plants under investigation. Among the detected fatty acids were move to after the each palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), each showing diverse proportions across different species. Additionally, minor fatty acids and branched-chain fatty acids were also identified, indicating the complexity of the lipid composition in Apiaceae plants. The fatty acid composition of some Apiaceae species expressed as percentage based on dry weight is presented in Table 2-3. The fixed oil content was highest in *A. orientalis* leaf ethanol extract (17.82%) while the lowest in *A. orientalis* leaf hexane extract (2.38%).

A wide spectrum of 27 fatty acids was confirmed, ranging from C6:0 to C22:6 in *A. orientalis* leaf and fruit fixed oils. Palmitic, oleic, linoleic and α- linoleic acid were the major fatty acids present in the *A. orientalis* fixed oils (Figure 1). However, in the fixed oil obtained from leaf of *A. orientalis* eicosadienoic acid was found to be the major fatty acid (51.048%).

The fatty acid composition of *F. asparagifolia* leaf and fruit part extracts are given in Table 3 and Figure 2. When the results were evaluated, the palmitic acid percentage of *F. asparagifolia* leaf ethanol and hexane extracts was found to be higher than other fatty acids (36.87% and 36.40%, respectively). Linoleic acid and oleic acid percentages were determined as 25.8% and 21.8% for hexane extract, respectively. For ethanol extract, it was determined as 24.52% and 21.65%, respectively. The linoleic acid percentage of *F. asparagifolia* fruit ethanol extract was found to be higher than other fatty acids (38.93%). The percentages of oleic acid and palmitic acid were determined as 21.80% and 36.40% for the leaf hexane extract, respectively. For ethanol extract, it was determined as 21.65% and 36.87%, respectively.

Palmitic acid (16:0) is a saturated fatty acid found in the diet and synthesized endogenously. It is an important component of cell membranes, secretion and transport lipids in our body (Agostoni et al., 2016). It has protective effects on obesity, Type 2 diabetes, cancer and cardiovascular diseases (Mancini et al., 2015). In our study, palmitic acid was found to be the major component in *F. asparagifolia* leaf and linoleic acid was identified as the predominant component in the fruit of the plant. Despite being in the same genus, as can be seen from the results, the amount of linoleic, palmitic and oleic acids was dominant in the ethanol and hexane extracts of *F. syriaca* species (Figure 3), and the amount of oleic acid (40.372% and 49.197%, respectively) was found to be significantly higher than that of *F. asparagifolia* species.

The composition of fatty acids in plants is determined by a combination of genetic factors, environmental conditions, and the stage of development. The presence of specific fatty acids in Apiaceae plants may contribute to their nutritional value, flavor profiles, and potential pharmacological properties. For example, omega-3 fatty acids such as linolenic acid has been linked to numerous health benefits, such as cardiovascular protection and anti-inflammatory effects.

The GC-FID analysis provided detailed information about the fatty acid profiles of Apiaceae plants, which can be valuable for quality control, nutritional assessment, and potential biotechnological applications (Hajib et al., 2023). Further studies could focus on exploring the biosynthesis pathways of unique fatty acids in these plants and investigating their functional roles in plant metabolism and adaptation.

4. Conclusion

In this study, fatty acid constituents of some Apiaceae species were determined by GC-FID. In conclusion, this study demonstrated the application of GC-FID for analyzing the fatty acid composition of Apiaceae plants, revealing a diverse array of fatty acids with potential implications for nutrition, flavor, and health. Exploring the lipid profiles of these plants can enhance their application in the food, pharmaceutical, and cosmetic industries, highlighting the importance of lipid analysis in plant science research. This was the first report on the study of fatty acid compounds from some Apiaceae family plants including *A. orientalis, F. asparagifolia* and *F. syriaca*.

CRediT author statement

NE & FA: Investigation, benchwork, study design, collection, analysis, and interpretation of data, and manuscript writing. YB: Investigation, benchwork, study design, collection, analysis, and interpretation of data, manuscript writing and supervision of the work. MRB & HK: Conceptualization, Benchwork, experimental design, data collection, and reviewing, editing of manuscript.

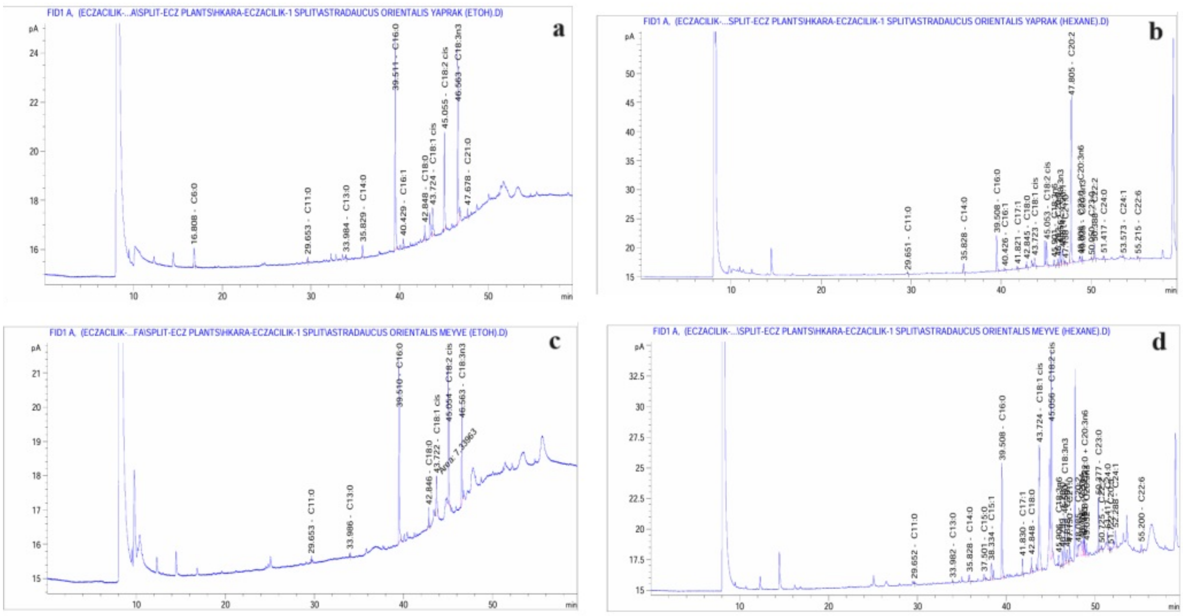

Figure 1. GC-FID chromatograms of ethanol and n-hexane extract of *Astrodaucus orientalis* leaves (a & b) and fruit (c & d).

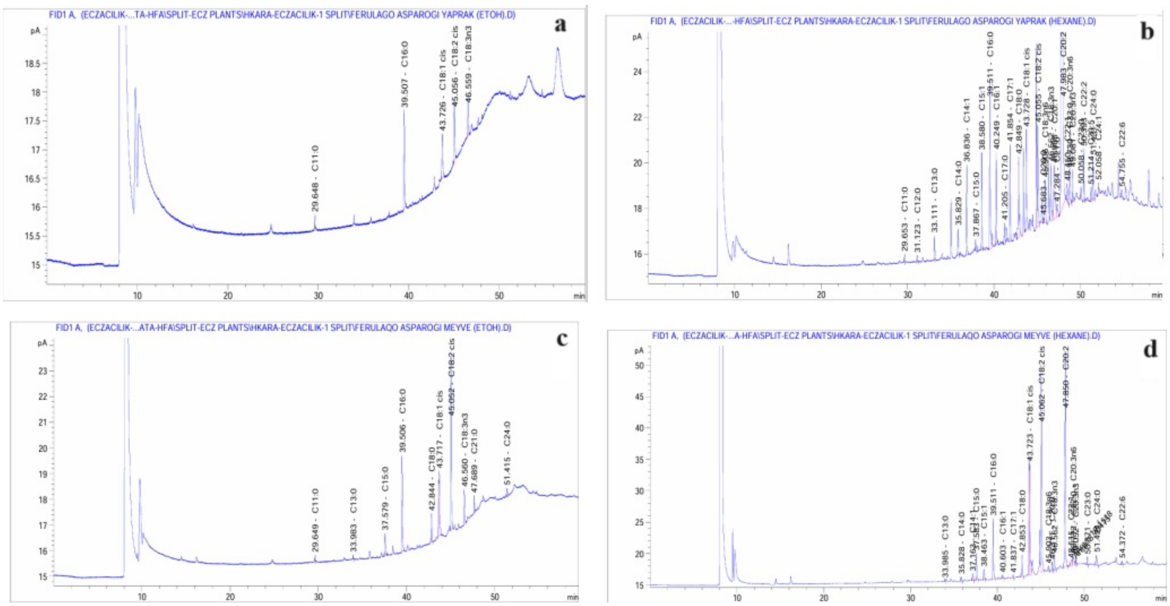

Figure 2. GC-FID chromatograms of ethanol and n-hexane extract of *Ferulago asparagifolia* leaf (a & b) and fruit (c & d).

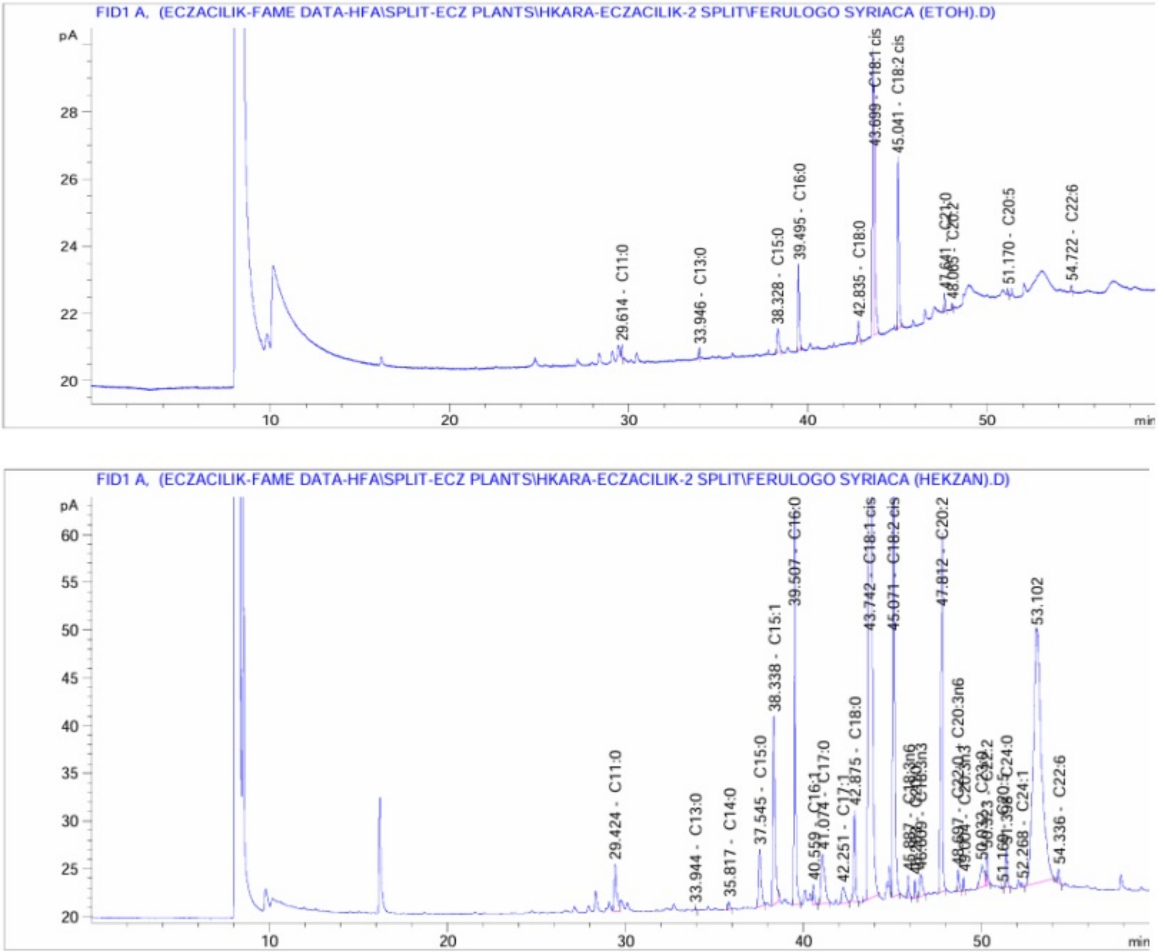

Figure 3. GC-FID chromatogram of *Ferulago syriaca* fruit ethanol and n-hexane extract.

Table 2. Fatty acid composition of hexane and ethanol extract of *Astrodaucus orientalis* leaf and fruit (%).

Fatty acids	*Astrodaucus orientalis Leaf*		*Astrodaucus orientalis Fruit*	
	Ethanol	Hexane	Ethanol	Hexane
Caproic acid (C6:0)	4.13	NDT	NDT	NDT
Undecanoic acid (C11:0)	0.48	0.179	0.561	0.133
Lauric acid (C12:0)	NDT	NDT	NDT	NDT
Tridecanoic acid (C13:0)	0.286	NDT	0.487	0.103
Myristic acid (C14:0)	2.141	2.598	NDT	0.736
Ginkgolic acid (C15:1)	NDT	NDT	NDT	2.661
Palmitic acid (C16:0)	35.969	9.814	37.649	12.760
Palmetoleic acid (C16:1)	1.495	NDT	NDT	NDT
Margaric acid (C17:1)	NDT	NDT	NDT	1.272
Stearic acid (C18:0)	2.397	1.568	3.846	1.581
Oleic acid (C18:1 cis)	6.189	3.201	9.524	22.549
Linoleic acid (C18:2 cis)	16.551	6.548	28.725	24.231
γ-linoleic acid (C18:3n6)	NDT	1.409	NDT	1.055
Arachidic acid (C20:0)	NDT	NDT	NDT	1.223
α- linoleic acid (C18:3n3)	29.289	5.149	19.206	5.276
Eicosenoic acid (C20:1)	NDT	6.920	NDT	2.231
Heneicosanoic acid (C21:0)	1.071	1.000	NDT	1.810
Eicosadienoic Acid (C20:2)	NDT	51.048	NDT	1.535
Eicosatetraenoic acid (C20:4)	NDT	NDT	NDT	3.293
Erucic acid (C22:1)	NDT	NDT	NDT	1.544
Behenic acid + γ-linoleic acid (C22:0 + C20:3n6)	NDT	1.231	NDT	3.193
Eicosatrienoic acid (C20:3n3)	NDT	1.043	NDT	1.284
Tricosanoic acid (C23:0)	NDT	0.436	NDT	4.333
Docosadienoic Acid (C22:2)	NDT	3.346	NDT	0.348
Lignoceric acid (C24:0)	NDT	NDT	NDT	2.253
Nervonic acid (C24:1)	NDT	0.542	NDT	2.756
Docosahexaenoic acid (C22:6)	NDT	0.769	NDT	0.958

NDT: not detected.

Table 3. Fatty acid composition of *Ferulago asparagifolia* and *Ferulago syriaca* extracts (%).

Fatty Acids	*Ferulago asparogifolia* Leaf		*Ferulago asparogifolia* Fruit		*Ferulago syriaca* Fruit	
	Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
Undecanoic acid (C11:0)	2.98	0.11	0.84	NDT	1.161	0.942
Lauric acid (C12:0)	NDT	0.13	NDT	NDT	NDT	NDT
Tridecanoic acid (C13:0)	NDT	0.50	0.44	0.049	0.891	0.016
Myristic acid (C14:0)	NDT	0.63	NDT	0.511	NDT	0.091
Pentadecanoic acid (C15:0)	NDT	NDT	NDT	3.724	5.840	1.199
Ginkgolic acid (C15:1)	NDT	NDT	NDT	1.337	NDT	3.441
Palmitic acid (C16:0)	36.87	36.40	21.095	NDT	14.834	4.903
Stearic acid (C18:0)	NDT	2.27	5.97	2.853	3.386	1.191
Oleic acid (C18:1 cis)	21.65	21.80	15.568	13.907	40.372	49.197
Linoleic acid (C18:2 trans)	NDT	NDT	NDT	NDT	NDT	NDT
Linoleic acid (C18:2 cis)	24.52	25.80	38.930	25.651	29.736	10.834
γ-linoleic acid (C18:3n6)	NDT	1.22	NDT	0.604	NDT	0.244
Alpha linoleic acid (C18:3n3)	13.98	1.69	7.415	2.004	NDT	0.488
Heneicosanoic acid (C21:0)	NDT	0.56	2.571	NDT	2.174	NDT
Eicosadienoic acid (C20:2)	NDT	NDT	NDT	32.658	0.413	5.414
Behenic acid + γ-linoleic acid (C22:0 + C20:3n6)	NDT	0.97	NDT	2.398	NDT	0.285
Eicosatrienoic acid (C20:3n3)	NDT	NDT	NDT	0.499	NDT	0.107
Erucic acid (C22:1)	NDT	0.44	NDT	1.217	NDT	NDT
Eicosapentaenoic acid (C20:5)	NDT	NDT	NDT	NDT	0.491	0.005
Tricosanoic acid (C23:0)	NDT	NDT	NDT	NDT	NDT	0.776
Lignoceric acid (C24:0)	NDT	1.01	1.716	1.16	NDT	0.461
Nervonic acid (C24:1)	NDT	0.34	NDT	NDT	NDT	0.05
Docosahexaenoic acid (C22:6)	NDT	NDT	NDT	NDT	0.702	0.191

NDT: not detected.

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