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Defne (*Laurus nobilis* L.) Tohumu, Meyvesi ve Meyve Kabuğundaki Yağ Asidi Profilinin GC-FID ile Belirlenmesi

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ÖZET

Laurus nobilis meyvesinin sabit yağı günümüzde gıda alanında koku, baharat ve çay olarak; sağlık alanında antifungal, antioksidan, antimikrobiyal gibi etkileriyle kullanılmaktadır. *Laurus nobilis* sabit yağı elde edilirken yağ eldesinde kullanılacak bitki kısımlarının ve yağ elde etme yönteminin yağ verimliliğini ve yağ asit profilleri üzerine etkisini değiştireceği düşünülmektedir. Bu nedenle bu çalışmada; *Laurus nobilis* meyvesi, meyve çekirdeği ve meyve kabuğu olarak bitkinin 3 farklı kısmı soğuk sıkım ve soxhlet yöntemleri ile ekstrakte edilmiş ve bunların yağ asit profilleri GC-FID ile analiz edilmiştir. Analiz sonucuna göre, yağ asitlerinin en yüksek konsantrasyonu, %50.71 doymuş yağ asitlerinden oluşan soxhlet yöntemiyle ekstrakte edilmiş defne meyvesine aittir. Bunu %49.78 oranında doymamış yağ asitleri içeren soğuk sıkım yöntemiyle elde edilmiş defne kabuğu takip etmektedir. Ardından soxhlet yöntemiyle ekstrakte edilmiş %40.40 oranında doymuş yağ asitlerinden oluşan defne tohumu gelmektedir. Bu çalışmada, defnenin meyve, meyve çekirdeği ve meyve kabuğu kısımlarındaki yağ asidi oranlarına farklı ekstraksiyon yöntemlerinin etkisi incelenmiştir.

Anahtar kelimeler: Defne, Doymuş yağ asitleri, Doymamış yağ asitleri, Gaz kromatografisi, *Laurus nobilis*

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Determination of Fatty Acid Profiles in Seed, Fruit and Fruit Peel Parts of the Laurel (*Laurus nobilis* L.) by GC-FID

ABSTRACT

The fixed oil of *Laurus nobilis* fruit is used as a fragrance, spice, and tea in the field of food today; and it is used in medicine with its various effects such as antifungal, antioxidant and antimicrobial. While obtaining *Laurus nobilis* fixed oil, it is presumed that different parts of the plant and the methods to be used in oil extraction will change the oil yield and the pharmaceutical effect of the oil. Therefore, in this study, three different parts of *Laurus nobilis* which are fruit, fruit seed and fruit peel were extracted by cold pressed and Soxhlet methods and their fatty acid profiles were analyzed by GC-FID. The results show that the highest concentration of fatty acids belong to the laurel fruit extracted by soxhlet method consisting of 50.71% saturated fatty acids. The laurel peel extracted by cold pressed method follows it containing unsaturated fatty acids with a rate of 49.78%. Then the laurel seed extracted by soxhlet method follows it consisting of 40.40% saturated fatty acids. In this study, the effect of different extraction methods on the fatty acid ratios in the fruit, fruit seed and fruit peel parts of laurel was examined.

Key words: Laurel, *Laurus nobilis,* Saturated fatty acid, Unsaturated fatty acid, Gas chromatography

1. INTRODUCTION

The laurel is a forest plant belonging to the *Lauraceae* family and its leaves remain fresh throughout the year. Many different parts of the plant such as fruit, seeds, and leaves have been used by many civilizations from past to present in various fields such as medicine, cosmetics, and food (Baytop, 1999). It is known that the plant, which is known to have more than 1000 species, is only one species in Turkey and is often grown on the Black Sea and Aegean coasts (Baydar, 2009). This species, named *Laurus nobilis*, is economically important in the southern region of the country, especially in the city of Hatay (Koçer et al., 2021). According to a study in 2014, Turkey is in the first place in the export ranking of laurel, which shows it is one of the important contribution values of the country economically.

The laurel leaves have a high antioxidant effect and is good for indigestion, and the tea obtained with the leaves has a regulatory effect for digestion and helps to maintain insulin balance (Kazeem et al., 2012). The essential and fatty acid contained in its fruit treat skin diseases such as eczema. It also relieves nervous pain and gives calmness to the body. As the fatty acid content in its seed, it is preferred to be used in many different fields such as medicine, food, and cosmetics. It was determined that it shows antimicrobial activity against specific gram-positive and gram-

negative bacteria (Fidan et al., 2019). In addition to that, studies have shown that essential oils obtained from leaves and fruits show anticancer activity against different types of cancer such as liver, colon, lung and kidney cancer (Saab et al., 2021; Anzano et al., 2022). It is known that 20 different fatty acids are obtained from the fruit of laurel. The main fatty acids amongst them are oleic, linoleic, palmitic, and lauric. These fatty acids can appear in two different types of fatty acids which are fixed and essential oils (Armijo et al., 2017).

Essential oils, which have a unique strong smell and aroma and are liquid at room temperature, can be obtained from various parts of plants such as leaves, flowers and fruits. Although they are insoluble in water, they can be obtained by steam distillation method. Besides, fatty acids, which can be found in liquid and solid form at room temperature, are obtained from the parts of fruits and seeds of the plant. Fixed oils, like essential oils, have a water-insoluble nonpolar structure. It is generally obtained by maceration method using certain organic solvents or by pressing methods such as hot and cold pressed. Today, the most preferred pressing method is cold pressed, which is a mechanical method, preventing contamination and preventing potential structural deformations that may occur under excessive temperature (Marzouki et al., 2008). Therefore, when oils are obtained in this way, they preserve their aroma and chemical structure (Kaseke et al., 2021). Another method is the solvent extraction method, which is the extraction of fatty acids by dissolving in solvents which have lower boiling points.

In this study, fatty acids were extracted from three parts of laurel plant as seed, fruit and fruit peel by soxhlet extraction method and cold pressed method. The fatty acid ratios in their contents were determined by using Gas Chromatography (GC) method. Gas chromatography flame ionization detector (GC-FID) is among the most frequently used gas chromatography detection methods. Flame ionization detectors respond to many hydrocarbons. In detail, flame ionization detectors use flame to ionize organic compounds. After the sample leaves the GC column, each analyte passes through an airless, hydrogenated flame that ionizes the carbon atoms. After the ionization process, the ions are collected and a current is created in the electrodes of the detector, and then measurements are obtained as electrical signals. By GC-FID, fatty acids extracted from seed, fruit, and fruit peel extracts of laurel plant were analyzed and those parts of the plant with high percentages among their components were determined as well as their fixed oil contents. Additionally, the ratios of the detected fixed oils were determined as saturated and unsaturated fatty acids.

2. EXPERIMENTAL

2.1. Plant materials

The *Laurus nobilis* plant to be used in the experiment was collected from the laurel tree in the garden of the University of Health Sciences, Hamidiye Faculty of Pharmacy, in Üsküdar, Istanbul. After the plant fruits were collected in December 2021, they were dried for about 2 months in a place away from the sun. After the laurel was dried, it was divided into 3 parts as laurel fruit, laurel fruit peel and laurel seed to use in the experiment.

2.2. Extraction

2.2.1. Soxhlet Method

The extraction was performed according to the method of Türkmen and Koçer (2021) with some modifications. Each of the plant parts ground with the grinding machine was extracted with hexane at a ratio of 1:10. Afterwards, hexane was placed in a glass flask for the soxhlet assembly. The heater temperature was brought above the boiling temperature of hexane (69°C). The evaporation and condensation cycle of the hexane solvent was repeated 15 times to extract oil from the sample with a maximum efficiency. Finally, oil, which was transferred to the glass balloon, was obtained by removing its solvent with an evaporator device.

2.2.1. Cold Pressed Method

A cylindrical expeller (11 mm) powered by a 6.1 kW electric motor was used to extract oil from the seed, peel and fruit of the laurel. The speed of the machine was kept constant during the pressing. A non-contact measurement device was used to ensure that the temperature of the machine did not rise above 25°C.

2.3. GC-FID Analysis

The analysis was performed according to the method of Akyüz et al. (2019) with some modifications. The extracted fatty acid components were converted to the methyl esters, which is called esterification, to transform them into their volatile forms for analysis of GC-FID. Hexane was used as the solvent in the GC analysis. The injection volume was set to 1 µL. The inlet pressure was maintained at 13.50 psi and the total flow at 14 mL per minute. The flow rate of (5%-phenyl)-methylpolysiloxane used as column filler was fixed at 1 mL per minute. The results were given as percentage (%) average and standard deviation of triplicate analysis.

2.4. Statistical Analysis

GraphPad Prism5 software was used to perform the statistical analysis. The analysis was performed using two-way ANOVA test (pairwise comparison test: Bonferroni) with

significances set at: ns: $p>0.05$ and ***$p<0.001$ as indicated.

3. RESULTS and DISCUSSION

Using two different extraction pathways which are soxhlet method and cold pressed method, fatty acids were extracted from different parts of *L. nobilis*. After that, fatty acid components were converted to their volatile derivatives which were methyl esters of fatty acids for analysis of GC-FID. The fatty acids were identified by comparison to the internal standards (Supelco 37 mix) and analyzed. The percentage of fatty acid concentrations were quantified by using the GC peak areas. The percentage of fatty acids which were obtained by soxhlet method were given in Table 1 and Figure 1. The percentage of fatty acids which were extracted by cold pressed method were presented in Table 2 and Figure 2.

Table 1. Percentage of fatty acid content of *L. nobilis* fixed oil extracted by soxhlet method, No detection: *ND*

RT*	Fatty Acid	Fruit Peel X̄±SD*	Fruit X̄±SD	Fruit Seed X̄±SD
6.18	*C6:0 (Caproic acid)*	*ND*	14.34 ± 0.02	16.72 ± 0.01
9.51	*C12:0 (Lauric acid)*	*ND*	22.75 ±0.03	12.59 ± 0.03
14.84	*C16:0 (Palmitic acid)*	4.62 ± 0.03	2.54 ± 0.02	6.06 ± 0.01
18.61	*C17:1 (Anandamide)*	*ND*	7.39 ± 0.02	3.09 ± 0.01
20.48	*C18:1n9c (Oleic acid)*	9.75 ± 0.02	10.88 ± 0.02	16.44 ± 0.03
22.28	*C18:2n6c (Linoleic acid)*	3.96 ± 0.02	8.24 ± 0.01	8.87 ± 0.02
23.29	*C20:0 (Arachidic acid)*	2.15 ± 0.02	9.20 ± 0.01	5.03 ± 0.02
23.95	*C18:3n6 (γ-Linolenic acid)*	*ND*	1.64 ± 0.02	*ND*
24.67	*C:21 (Heneicosylic acid)*	*ND*	1.89 ± 0.01	*ND*
27.96	*C22:1 (cis-13-Erucic acid)*	*ND*	3.34 ± 0.02	*ND*
Saturated fatty acid		6.77	50.71	40.40
Unsaturated fatty acid		13.71	31.50	28.41

* Retention time: RT, Standard deviation: SD

Ten different fatty acids were detected and quantified in the oil extracted by soxhlet method. The highest concentration belonged to lauric acid with 22.75% in the extract of the

fruit part. The second highest concentration belonged to caproic acid with 16.72% in the extract of the seed part. Then oleic acid follows with 16.44% in the extract of the seed part. The lowest concentration belonged to ɣ-Linolenic acid with 1.637% which was found in the fruit part. The second lowest concentration belonged to heneicosylic acid with 1.89% in the extract of fruit part. Then arachidic acid follows with 2.15% in the extract of peel part. According to the statistical data, there was no significant difference between the values given in Table 1 and Figure 1.

The percentage of saturated and unsaturated fatty acids extracted from three different parts of laurel by soxhlet method were evaluated (Table 1). The highest percentage of saturated fatty acid belong to the fruit part of laurel with 50.71%. The highest percentage of unsaturated fatty acid also belong to the fruit part of laurel with 31.50%. Another study confirmed that the percentage of the saturated fatty acids which were extracted from *L. nobilis* fruits was 34.8% and the percentage of the unsaturated fatty acids was 62.4% (Nourbakhsh et al., 2005). In that study, the fruits were collected from Izmir, a coastal city of Turkey, and extracted by soxhlet method performed by using petroleum ether while the fatty acids in this study were extracted by soxhlet method performed by using hexane. This can show that the solvent chosen in the extraction method can be significant to achieve an optimum percentage of specific fatty acids.

Table 2. Percentage of fatty acid composition of *L. nobilis* fixed oil extracted by the cold pressed method, No detection: *ND*

RT	**Fatty Acid**	**Fruit Peel** $\bar{X}$**±SD**	**Fruit** $\bar{X}$**±SD**	**Fruit Seed** $\bar{X}$**±SD**
6.18	*C6:0 (Caproic acid)*	22.10± 0.02	21.97± 0.02	12.74± 0.19
9.51	*C12:0 (Lauric acid)*	*ND*	3.84± 0.02	6.77± 0.02
14.84	*C16:0 (Palmitic acid)*	8.49± 0.34	2.81± 0.01	3.34± 0.03
20.48	*C18:1n9c (Oleic acid)*	33.32± 0.01	13.48± 0.02	9.05± 0.02
22.28	*C18:2n6c (Linoleic acid)*	16.45± 0.02	6.03± 0.01	6.48± 0.02
23.29	*C20:0 (Arachidic acid)*	*ND*	2.69± 0.01	*ND*
Saturated fatty acid		30.79	31.30	22.97
Unsaturated fatty acid		49.78	19.51	15.51

Six different fatty acids were detected and quantified in the oil extracted by cold pressed method. The highest concentration belonged to oleic acid in the extract of the peel part with 33.32%. The second highest concentration belonged to caproic acid in the extract of the peel part with 22.10%. Then caproic acid in the extract of fruit part follows with 16.44%. The lowest concentration belonged to arachidic acid with 2.69% which was found in the fruit part. The second lowest concentration belonged to palmitic acid with 2.81% in the extract of fruit part. Then palmitic acid in the extract of the seed part follows with 3.34%. According to the statistical data, there was no significant difference between the values given in Table 2 and Figure 2.

The percentage of saturated and unsaturated fatty acids extracted from three different parts of laurel by cold pressed method were evaluated (Table 2). The highest percentage of saturated fatty acid belong to the fruit part of laurel with 31.30% and the peel part follows with 30.79%. Also, the highest percentage of unsaturated fatty acid belong to the peel part of laurel with 49.78%.

In another study, the *L. nobilis* fruits were collected from Kesab located in Syria and the fatty acids were extracted by maceration method (Said et al., 2018). When the GC analysis results of the study of Said et al. (2018) and of this study are examined, the components of fatty acids extracted from laurel fruits can be listed as oleic acid (48.7-10.9%, respectively), linoleic acid (17.0-8.2%, respectively), palmitic acid (16.6-2.5%, respectively), lauric acid (14.0-22.8%, respectively). The difference in percentages could be attributed to the geographical factors such as temperature difference, precipitation amounts, sea level difference, and especially solar radiation since it is examined by some studies to be very essential for plant growth and its fatty acid concentration (Echarte et al., 2013). The extraction method in this study is soxhlet method by using hexane and the method of Said et al. (2018) is maceration with petroleum ether. Therefore, it cannot be directly said that the difference in percentages could be attributed to the extraction method, or the solvent used in the extraction since there are two variable parameters. Besides, a study which examined the fatty acid profiles of para rubber seed oils extracted with hexane and petroleum ether by methods of soxhlet and maceration shows that the fatty acid compositions are not very variable regardless of the extraction method or the solvent used (Raknam et al., 2017).

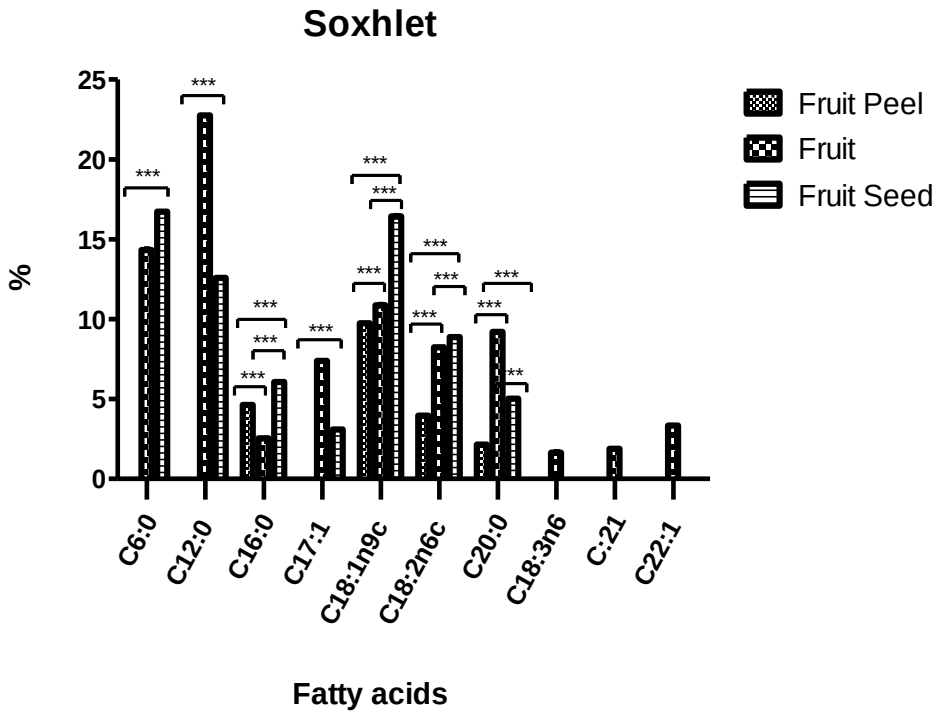

Figure 1. Ratios of fatty acids in three parts of laurel extracted by soxhlet method (***p<0.001)

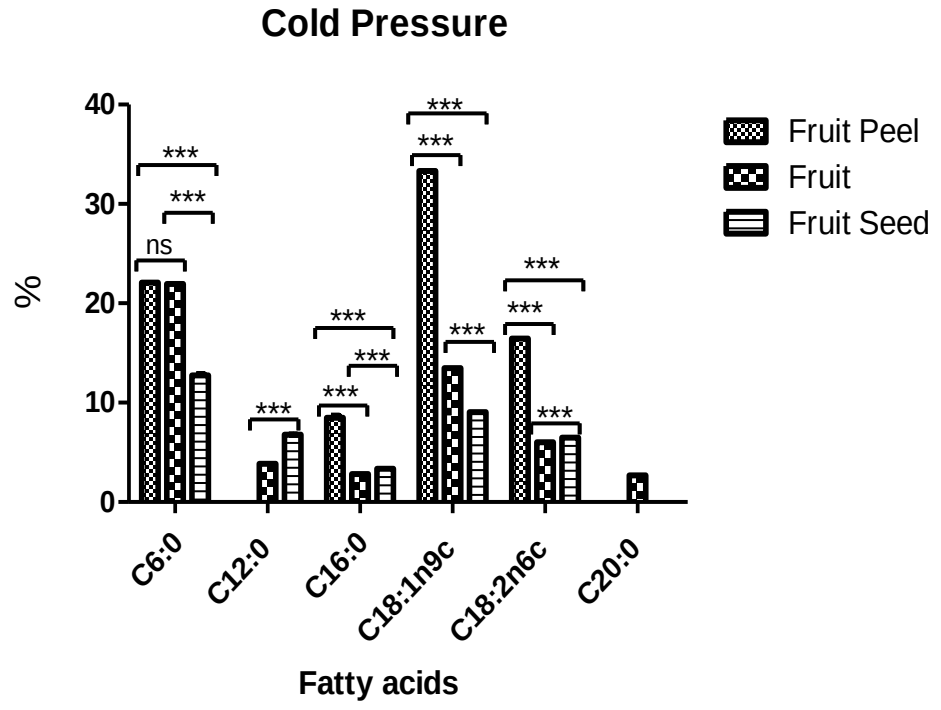

Figure 2. Ratios of fatty acids in three parts of laurel extracted by cold pressing method (***p<0.001, ^{ns} p>0.05)

The data of percentages given in two tables were compared to each other for the comparison of the methods applied to three parts of the laurel. It was seen that the fatty acid concentrations detected in different parts of *L. nobilis* had completely different values when performed with different methods. The lauric acid extracted from the fruit part by soxhlet method was detected as 22.75% while that fatty acid was determined as 3.84% in the oil extracted by cold pressed method. The higher concentrations of the fatty acids in the laurel fruits extracted by soxhlet method can indicate that soxhlet method is more efficient than cold pressed method for fruit part of the laurel. This indication is supported by a study performed by Türkmen et al. (2021).

As for the seed part, all the fatty acids extracted by soxhlet method had higher concentrations than the fatty acids obtained by cold pressed method. The results indicate that the most efficient extraction method for the seed of *L. nobilis* is also soxhlet method.

On the other hand, the caproic acid extracted from the fruit peel by cold pressed method was detected as 22.10% while that fatty acid obtained by soxhlet method could not be detected in the GC analysis. The arachidic acid extracted from the fruit peel by soxhlet method was detected as 2.15% while that fatty acid obtained by cold pressed method could not be detected in the GC analysis. It can be concluded that there is no preferred method for the peel part of laurel. It can be suggested for the future studies that the proper method for the extraction of peel part of *L. nobilis* should be chosen according to the specific fatty acid which is under the study.

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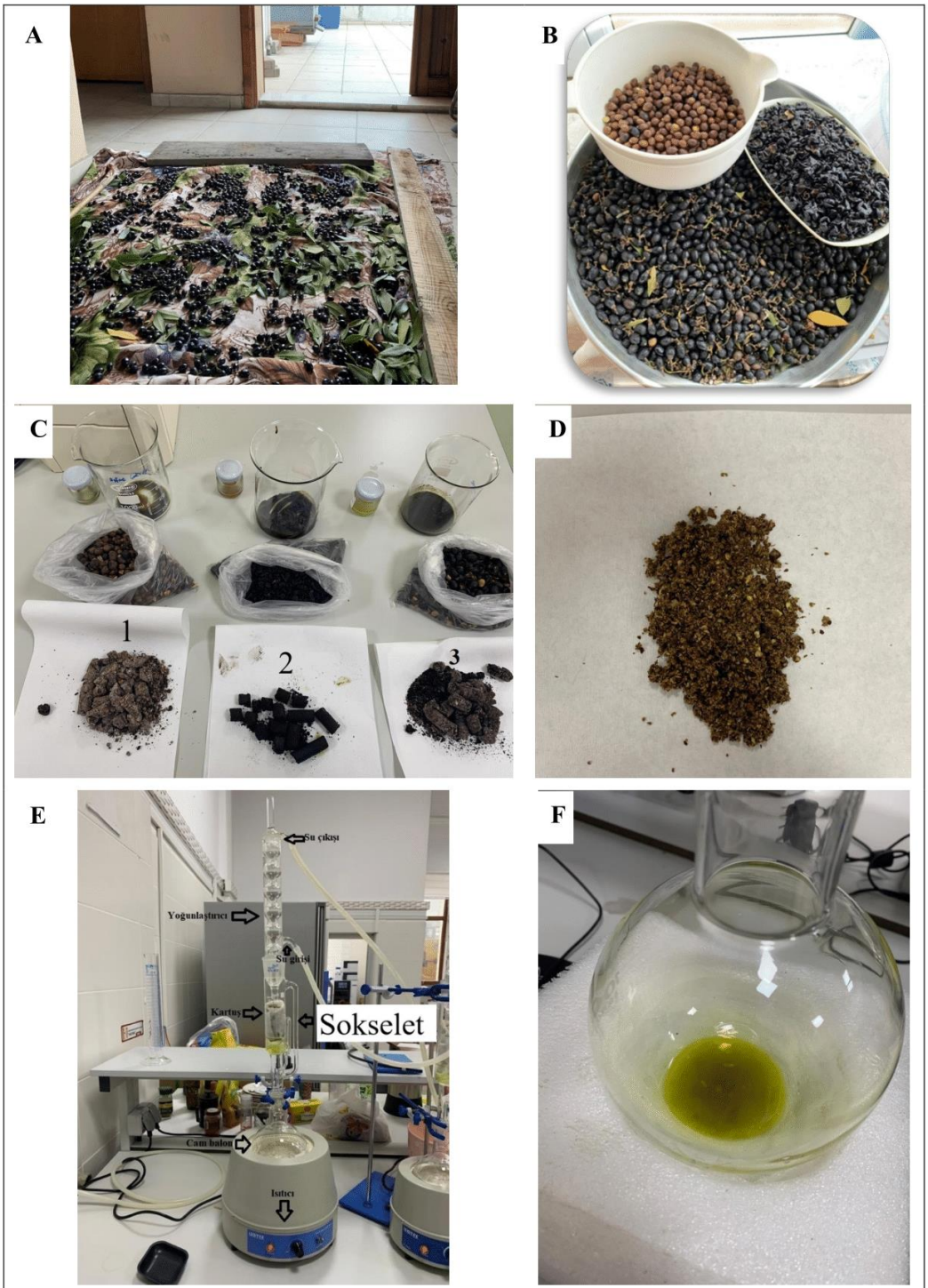
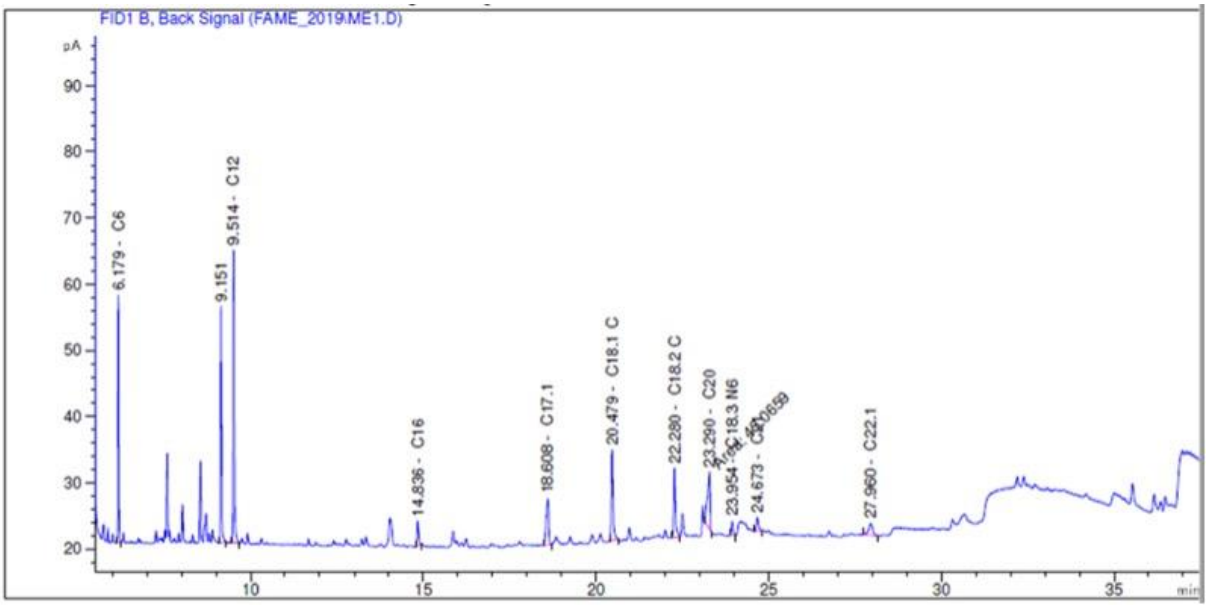
SUPPLEMENTARY MATERIALS for Determination of Fatty Acid Profiles in Seed, Fruit and Fruit Peel Parts of the Laurel (*Laurus nobilis* L.) by GC-FID



(**A**) Laurel fruits left to be dried. (**B**) Division of the laurel plant as seed, peel, and fruit. (**C**) Extraction of the laurel seed (1), peel (2) and fruit (3) oils by cold pressed method. (**D**) Grounded laurel seed to be used for Soxhlet extraction. (**E**) Extraction of the laurel seed, peel and fruit oils by Soxhlet method. (**F**) Extracted laurel seed oil by Soxhlet method after the evaporation of the solvent.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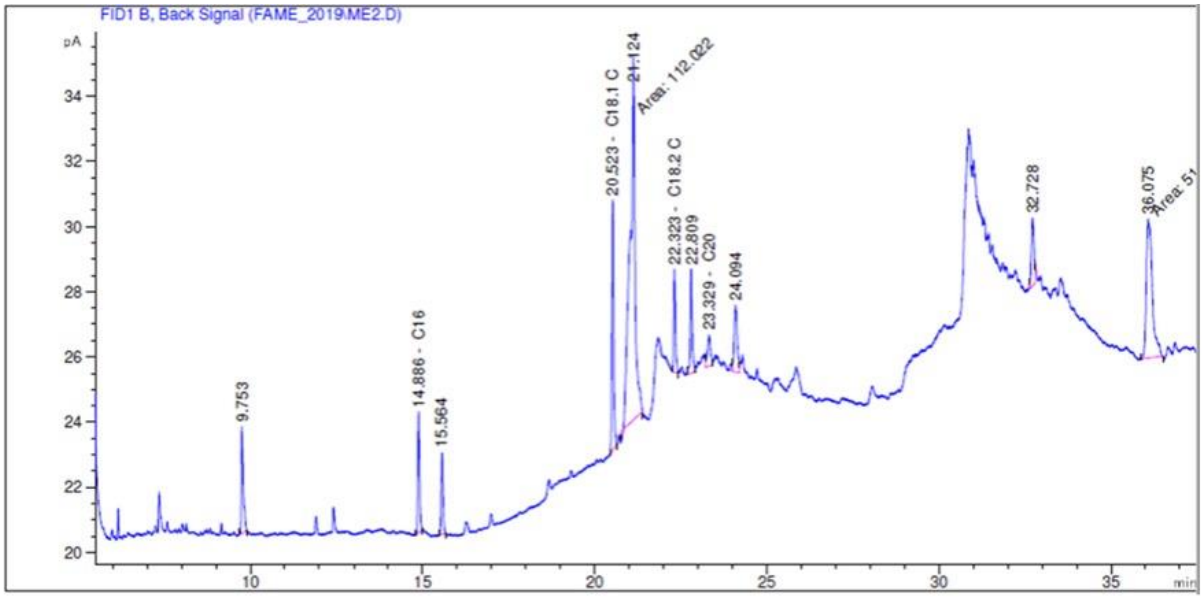
9.514 - C12
14.836 - C16
18.608 - C17.1
20.479 - C18.1 C
22.280 - C18.2 C
23.290 - C20
24.673 - C20:3
27.960 - C22.1
min

Pic1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22.809
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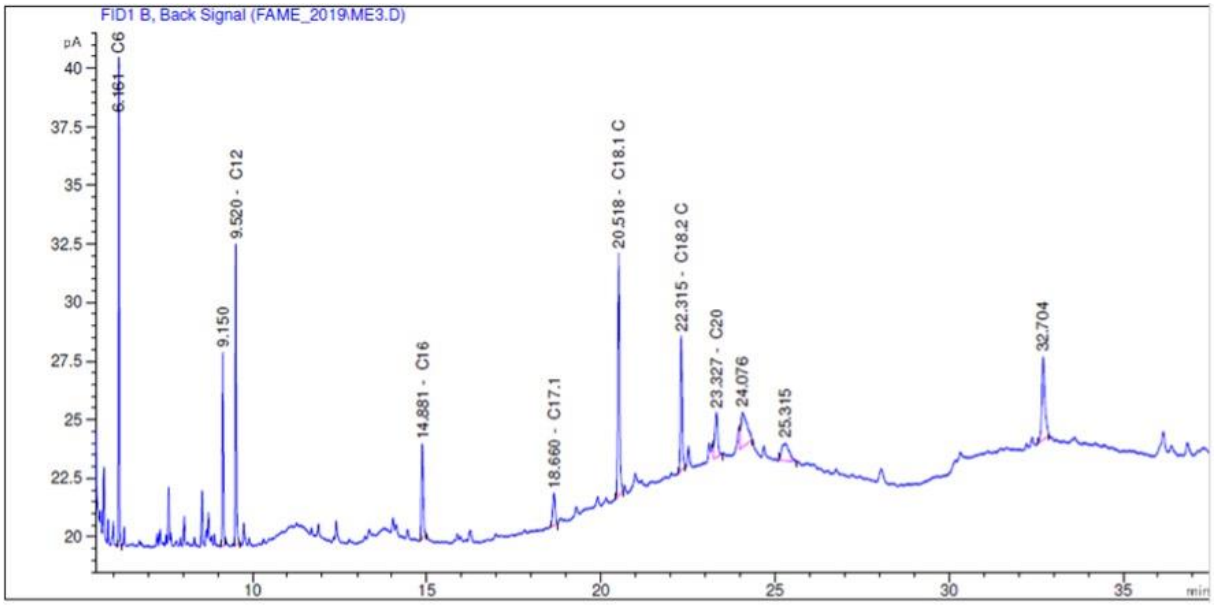
32.728
36.075
Area: 51
min

Pic2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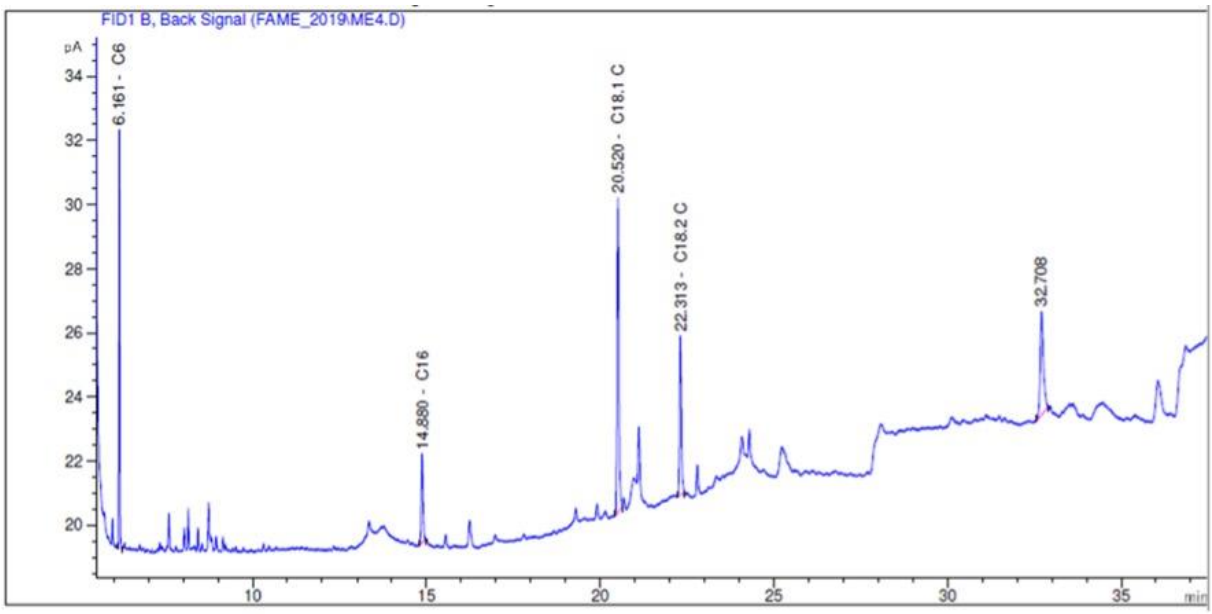
min

Pic3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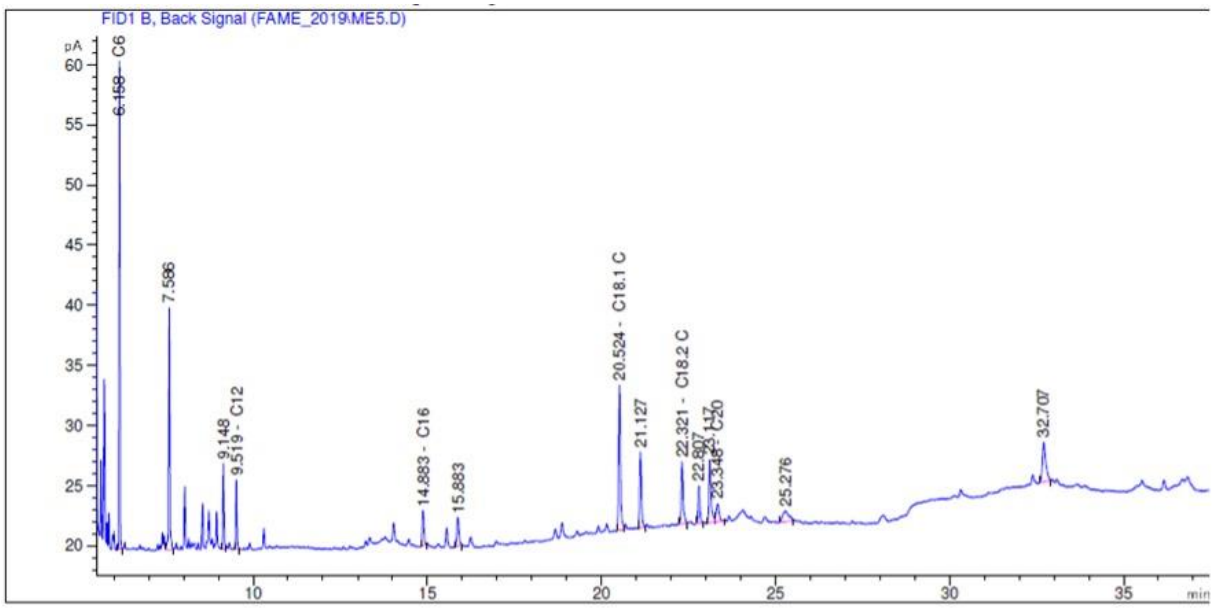
min

Pic4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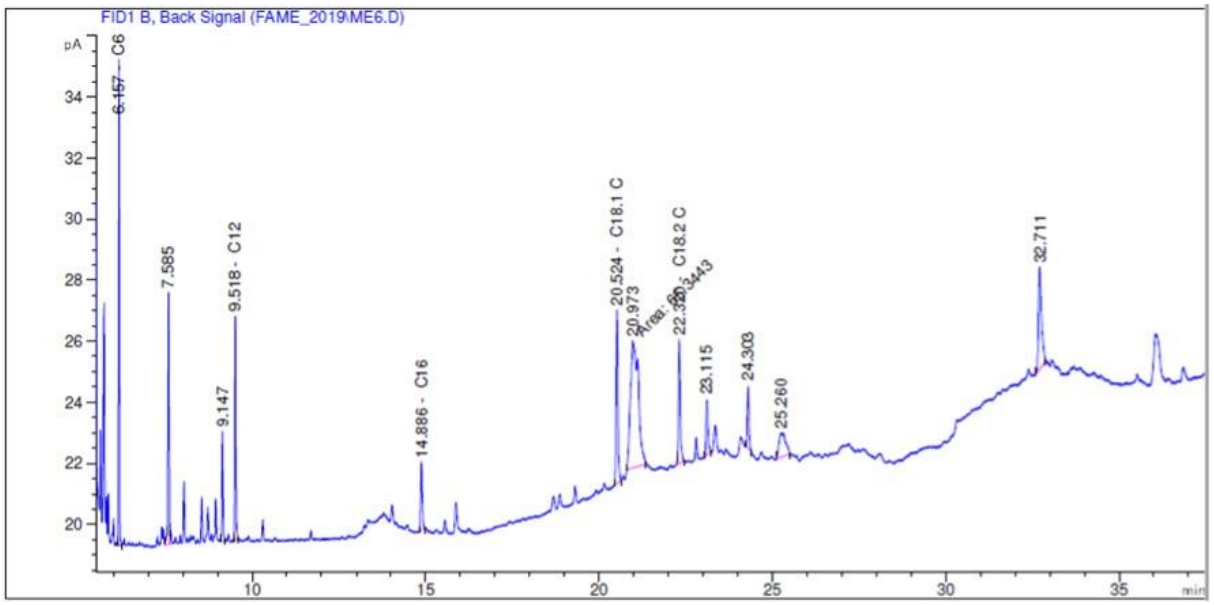
20.524 - C18.1 C
21.127
22.321 - C18.2 C
25.276
32.707
min

Pic5


FID1 B, Back Signal (FAME_2019\ME6.D)
pA
6.157 - C6
7.585
9.147
9.518 - C12
14.886 - C16
20.524 - C18.1 C
20.973
22.321 - C18.2 C
23.115
24.303
25.260
32.711
min

Pic6