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Evaluation of Supercritical Carbon Dioxide Extraction Method and Some Conventional Extraction Methods for The Determination of Fatty Acid Content from *Peganum harmala* L. Seed

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Abstract

The seeds of *Peganum harmala* L. (Syrian rue) (Zygophyllaceae) are commonly known to be "Harmal" or "Uzerlik" among the Turkish people. This study aims to compare with both SC-CO_2 extraction (at constant temperature conditions of 45ºC at 100, 200, 300 and 400 bar pressure) and some conventional extraction methods (ethanol, hexane, and dimethyl ether), and investigate the effects of extracting pressure parameters on SC-CO_2 extraction. As a result of the studies; it has been concluded that the SC-CO_2 extraction method is very advantageous in obtaining fatty acids from the *P.harmala* L. seed and that the pressure and temperature calibrations of the SC-CO_2 extraction device must be performed. It has been observed that a pressure of 400 bar is suitable for obtaining the highest fatty acid extracts of (C15:0), (C16:1), (C17:0), (C18:3) and saturated fatty, while a pressure of 100 bar is suitable for obtaining the highest fatty acid extracts such as (C14:0), (C18:1), and (C18:2).

Key words: *Peganum harmala* L., supercritical CO_2 extraction, fatty acid content.

Peganum harmala L. Tohumundan Yağ Asidi İçeriğinin Belirlenmesi için Süperkritik Karbondioksit Ekstraksiyon Yöntemi ve Bazı Geleneksel Ekstraksiyon Yöntemlerinin Karşılaştırılması

Özet

Türk halkı arasında *Peganum harmala* L. (Suriye rue) (Zygophyllaceae) tohumlarının yaygın olarak "Harmal" veya "Uzerlik" olduğu bilinmektedir. Bu çalışmanın amacı, SC-CO_2 ekstraksiyonu ile (100 ° C, 200, 300 ve 400 bar basınçta sabit sıcaklık koşullarında) ve bazı geleneksel ekstraksiyon yöntemleriyle (etanol, heksan ve dimetil eter) karşılaştırmak ve süperkritik ektraksiyon yönteminde basıncın etkisini araştırmaktır. Yapılan çalışmalar sonucunda; SC-CO2 ekstraksiyon yönteminin *P.harmala* L. tohumundan yağ asitlerinin elde edilmesinde çok avantajlı olduğu ve SC-CO_2 ekstraksiyon cihazının basınç ve sıcaklık kalibrasyonlarının yapılması gerektiği sonucuna varılmıştır. En yüksek (C15: 0), (C16: 1), (C17: 0), (C18: 3) ve doymuş yağ oranının elde edilmesi için 400 bar basınç en uygun iken; en yüksek (C14: 0), (C18: 1) ve (C18: 2) elde edilmesi için 100 bar basıncın, en uygun olduğu görülmüştür.

Anahtar kelimeler: *Peganum harmala* L., superkritik CO_2 ektraksiyon, yağ asidi kompozisyonu.

Introduction

Aromatic and medical plants constitute the raw material of many industries such as the pharmaceutical industry, cosmetics, perfume, soap, gum, and sugar. Many of the natural substances used as medicines are also derived from plants. Today, the production and use of non-natural drugs reached the highest level with the phase reached by chemistry. However, side effects resulting from the use of these drugs have led to an increase in demand for natural medicines in recent years with the development of natural life consciousness in humans. The demand for herbal medicines in the world and especially in European countries is increasing rapidly. The leading countries are Germany, France, Italy, England, and Spain. The use of natural herbal remedies in Asian countries has been very high compared to European countries since ancient times. Most biotechnology firms in Europe and the US are increasing their activities in the herbal plant sector. Anatolia has three phytogeographical regions (Europe-Siberia, Mediterranean and Iran-Turan). This position has resulted in a high number of endemic species and high plant diversity (Browicz, 1982). One-third of the species of Turkish flora are aromatic plants, and they are the main sources of essential oils (De Silva, 1995). Extraction is a separation process in which the desired substance to be separated from a mixture made by dissolving with a solvent in which the substance of interest is having different solvency against a compound which does not interfere with and which is to be separated. Two different solvents are used to accomplish this, while the other compounds pass to the second solvent. Extraction can be done several times to completely separate the desired compound (Güngör, 2000).

Concrete, absolute, pomat, resinoid, extract, carbon dioxide, microwave, ınfusion, tincture, oleoresin extraction methods are applied (Öztekin and Soysal, 1998). Stationary oils are a storage material in plants, especially in seeds. Fixed oils, natural vegetable or animal oils that are not volatile can be extracted with solvents such as petroleum ether, hexane, and trichloroethylene, or by extrusion or supercritical extraction without solvent (Sakar and Tanker, 1991). Essential oils are found mostly in the volatile fat cells of the inner tissues of the secretory follicles and the secretory pockets of the plant.

A substance on the temperature and pressure critical point is called "supercritical fluid". The critical point refers to the maximum temperature and the pressure at which the vapor and liquid phases can be in equilibrium. There is no phase separation due to the pressure increase, and a single phase is formed when there are two phases under the critical point. Supercritical fluids can be defined as fluids whose properties can be easily adjusted by pressure and temperature changes. They are similar to gases due to their low viscosity and high diffusiveness characteristics, but to liquids with high density and solubility properties.

The supercritical region offers more flexible solutions for use compared to conventional solvents with small changes in extraction conditions since it is a function of dissolution power density (Akgün and Akgün 2006).

CO_2 dissolves apolar substances because it is an apolar substance. Low molecular weight hydrocarbons and lipophilic organic compounds (ethers, esters) are easily extracted. Pressure and amount of modifier in the extraction with supercritical fluids (SC) method are the most important parameters affecting the affecting compound. In this method, the diffusion control model is a mass transfer model based on Fick's second law (Campos et al., 2005; Esquivel et al., 1999) and as the pressure increases, the solvent-solute interaction increases due to the increase in CO_2 concentration. The solvent strength of a supercritical fluid can be improved by changing the pressure and temperature with less importance. As the temperature increases over the threshold pressure, the solubility increases (Murga et al., 2000). The structure of the matrix on extraction efficiency with supercritical fluids has great importance. The relation of the matrix to the component to be extracted depends on the modification of the matrix, which will increase the mass transfer rate due to the swelling of the matrix.

Even if a 10-20% modifier is used around the critical point, the extraction profile changes very large and the percentage of polar components increases (Hamburger et al., 2004). By optimizing the extraction conditions in this method, desired phenolic components can be extracted with great success in one step. The greatest advantage of the method is that it prevents the deterioration of the component because there is no light and oxygen in the environment and that the antioxidant power can be obtained higher than in conventional methods.

The main advantages of the extract are that they do not contain any solvent residues, remain intact when exposed to high temperatures, can be extruded in a single step and reused without loss of solvent (William et al., 1996).

P. harmala L., a species belonging to the family Zygophyllaceae, is naturally grown in Central Anatolia (Tanker et al., 2007). It is commonly known with the names of the amulet, evil eye, wild pearl grass, mahmur flower, hamet and harmel among the people. The plant is generally 70 cm long, hairless, partly leafy, white flowering, perennial and bushy (Baytop, 1999).

In this study, the extraction of volatile oils from *P. harmala* L. seeds was compared with both SC-CO_2 extraction (at constant temperature conditions of 45ºC at 100, 200, 300 and 400 bar pressure) and some conventional extraction methods (ethanol, hexane, and dimethyl ether), and the effects of extracting pressure parameters on SC-CO_2 extraction were also investigated.

Material and Method

Biological Material

Mature seeds were collected from Altınakar location (37.752252, 40.422138) in Çınar district of Diyarbakır province on 10/15/2017. The dried seeds were used as plant material. Extraction studies were carried out in the Extraction Laboratory in the Directorate of Research and Application Center of Medical and Aromatic Plants of Dicle University.

SC-CO_2 Extraction Study

SC-CO_2 extraction was carried out in a Qualitec device, in 500 mL extractors (Fig.1). The SC-CO_2 extraction study was carried out at a constant temperature of 45 °C. In the SC-CO_2 extraction study, 100, 200, 300 and 400 bar pressure conditions were made separately and extracts were obtained.

Conventional Extraction Study

Ethanol, hexane and dimethyl ether were used as a solvent in some conventional extraction methods. The herbal material was left in the solvent for 24 hours. After the waiting period, the extraction was carried out using a vacuum rotary evaporator. The obtained extract was allowed to stand in the dark and at room temperature to remove the remaining solvent and concentrate it.

Fatty Acid Analysis

Total lipid extraction was performed according to the method of Bligh and Dyer (Bligh and Dyer, 1959). A chloroform and methanol mixture (2:1, vol/vol) was used as a solvent. Samples containing muscle lipid were transesterified with acidified methanol. Fatty acid methyl esters (FAMEs) were extracted with hexane and analyzed via capillary gas chromatography using a SHIMADZU GC 2010 PLUS equipped (laboratory of Dicle University Science Faculty) with a flame ionization detector (FID) and a fused silica capillary column (DB-23 (Bonded 50 % cyanopropyl), 30 m × 0.25 mm i.d.; 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA). The carrier gas was hydrogen (flow rate 30 mL/min), helium (flow rate 0.5 mL/min), dry air (flow rate 400 mL/min), and the split ratio was 1:20. The temperature profiles were as follows: initial temperature, 170 °C (initial time, 2 min); heating rate, 2 °C/min; final temperature, 210 °C. The FAMEs were identified using retention times compared with those of standard purified fatty acids (Sigma Chemical Co., St. Louis, MO, USA). Results were expressed as FID response area relative. Chromatograms of fatty acids methyl esters and total fatty acids quantities were obtained using GC Solution (Version 2.4) computer program. Peaks in the chromatogram of the analyzed samples were identified by comparison with the retention times of methyl esters of all fatty acids in the standard. The results are given as a qualitative value over% fatty acid.

Data Analysis

The data obtained from the study were subjected to statistical analysis in the statistical program JMP 7.0 (Copyright © 1989 - 2002 SAS Institute Inc.). The extraction methods were compared with each other according to the F test. In the study, $LSD_{0.05}$ test (multiple comparison tests) was used. Correlation and regression analysis were performed by obtained fatty acids compositions and the pressure used in the SC-CO_2 extraction method. The relationship between pressure and fat acid is examined.

Results and Discussion

In this study, mean values of the fatty acid composition obtained by different extraction methods from *Pegamum harmala* L. seeds and $LSD_{0.05}$ test groups of these values are given in Table 1.

Mean values for myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) were 9,63%, 2,05%, 24,03%, 0,65%, 0,84%, 10,2%, 27,77%, 23,69%, 1,1%, respectively. Myristic acid (C14:0) was 29.32% in SC-CO_2 extraction with a maximum pressure of 100 bar but at most 8.05% in the conventional methods. Pentadecanoic acid (C15:0), palmitoleic acid (C16:1), stearic acid (C18:0), linolenic acid (C18:3) were in SC-CO_2 extraction with a maximum pressure of 400 bar. However, these fatty acids are less obtained in conventional extraction methods. Palmitic acid (C16:0) was 30.84% in SC-CO_2 extraction with a maximum pressure of 200 bar but at most 27.02% in the conventional methods. Heptadecanoic acid (C17:0), oleic acid (C18:1), and linoleic acid (C18:2) were obtained from conventional extraction methods in the most amount of 1.17% (ethanol), 30.15% (ethanol), 47.57 (dimethyl ether), respectively. The highest unsaturated fatty acids were obtained by dimethyl ether extraction method from conventional methods while saturated fatty acids were obtained from SC-CO_2 extraction method at 400 bar pressure. While the sat./unsat. ratio in conventional extraction methods is not greater than 1, the sat./unsat. ratio in SC-CO_2 extraction method is larger than 1 at all pressures. Especially in the SC-CO_2 extraction method, as the pressure increases, the increase in the ratio sat./unsat. becomes remarkable (Table 1). Our findings are similar to the findings obtained by Küsmenoğlu (1996), Moussa and Almaghrabi (2016), Hassani and Hadek (2013).

Negative correlation between pressure and C14:0 (r=-0.64*), C18:1 (r=-0.95**), C18:2 (r=-0.84**) was found in SC-CO_2 extraction method. On the other hand, positive correlation was found between pressure and C15:0 (r=+0.99**), C16:0 (r=+0.73**), C16:1 (r=+0.95**), C17:0 (r=+0.59*), C18:0 (r=+0.82**), C18:3 (r=+0.84**) in SC-CO_2 extraction method. In the SC-CO_2 extraction method, it was found that the saturation fatty acids increased (r=+0.83**) while the unsaturated fatty acids decreased (r=-0.83**) while the pressure increased.

The relationship between fatty acids extracted by the SC-CO_2 method from *Peganum harmala* L. seeds and pressure is given in Fig.2-11.

A linear relationship between (C15:0), (C16:1), (C17:0), (C18:1), (C18:2), (C18:3), saturated and pressure was found, while a quadratic relationship was found between (C14:0), (C16:0), and (C18:0) acids (Fig.2-11). (C15:0), (C16:1), (C17:0), (C18:3) and saturated fatty acids were determined as 400 bar (Fig.3, Fig.5, Fig.6, Fig.10, and Fig.11), while the optimum pressure for (C14:0), (C18:1), and (C18:2) fatty acids was determined as 100 bar (Fig.2, Fig.8, Fig.9). In addition, the optimum pressure bar for (C16:0), (C18:0) fatty acids was determined to be 300 bar (Fig.4 and fig.7).

Table 1. Mean values of the fatty acid composition obtained

	Conventional Methods			SC-CO_2 Method				
Constituents	**Dimethyl ether**	**Ethanol**	**Hexan**	**100 b**	**200 b**	**300 b**	**400 b**	**Means**
(C14:0)	0.91 f	8.05 d	0.76 g	29.32 a	7.1 e	9.45 c	11.81 b	**9.63**
(C15:0)	0.52 g	2.45 c	0.86 f	1.84 e	2.43 d	2.89 b	3.35 a	**2.05**
(C16:0)	14.66 g	27.02 d	17.02 f	18.56 e	30.84 a	30.32 b	29.78 c	**24.03**
(C16:1)	0.16 g	0.85 c	0.25 f	0.46 e	0.83 d	0.94 b	1.05 a	**0.65**
(C17:0)	0.41 g	1.17 a	0.55 f	0.6 e	1.11 b	1.04 c	0.97 d	**0.84**
(C18:0)	6.00 g	11.32 d	6.14 e	6.06 f	13.63 c	13.96 b	14.3 a	**10.20**
(C18:1)	28.59 c	30.15 a	29.96 b	27.12 d	26.42 e	26.19 f	25.96 g	**27.77**
(C18:2)	47.57 a	18.13 c	43.48 b	15.13 e	16.30 d	13.84 f	11.38 g	**23.69**
(C18:3)	1.15 c	0.81 f	0.94 d	0.86 e	1.29 b	1.32 ab	1.36 a	**1.10**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Sat.	22.51 g	50.03 e	25.34 f	56.41 c	55.13 d	57.68 b	60.24 a	**46.76**
Unsat.	77.48 a	49.95 c	74.65 b	43.58 e	44.87 d	42.31 f	39.76 g	**53.23**
Sat./Unsat.	0.29 g	1.00 e	0.33 f	1.29 c	1.22 d	1.36 b	1.51 a	**1.00**

(C14:0): Myristic acid; (C15:0): Pentadecanoic acid; (C16:0): Palmitic acid; (C16:1): Palmitoleic acid ; (C17:0): Heptadecanoic acid; (C18:0): Stearic acid; (C18:1): Oleic acid; (C18:2): Linoleic acid; (C18:3): Linolenic acid; Sat: Saturated; Unsat: Unsaturated.

Note: Different letters between susceptible and resistant cultivars denote significant differences (LSD test, $p<0.05$).

Conclusions

It has been concluded that the SC-CO_2 extraction method is very advantageous in obtaining fatty acids from the *P.harmala* seed and that the pressure and temperature calibrations of the SC-CO_2 extraction device must be performed. As a results of this study it has been observed that a pressure of 400 bar is suitable for obtaining the highest fatty acid extracts of (C15:0), (C16:1), (C18:0), (C18:3) and saturated fatty, while the pressure of 100 bar is the most suitable for other pressure applications for obtaining (C14: 0), (C18: 1) and (C18: 2) fatty acid extracts.

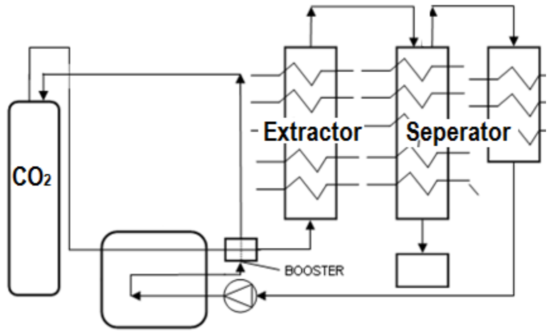


Figure 1a. The SC-CO_2 Pilot plant schematic flow

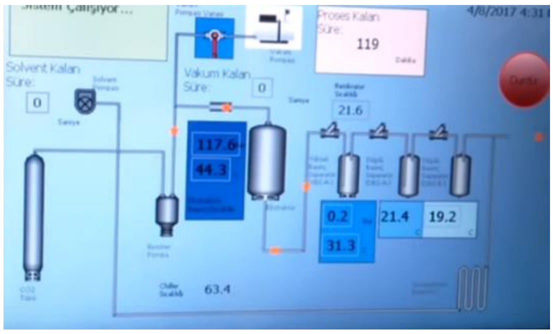
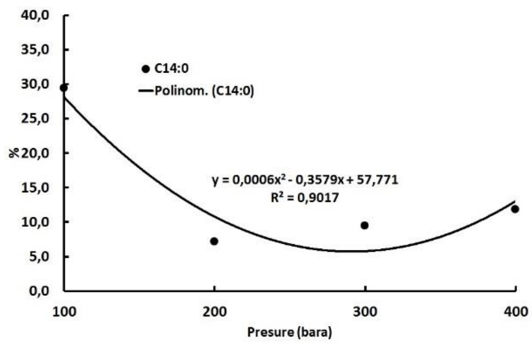


Figure 1b. The Qualitec SC-CO_2 device and control unit



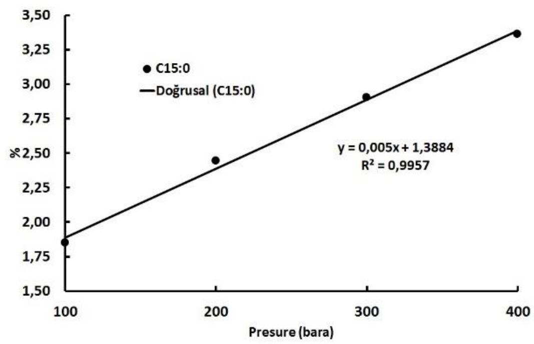
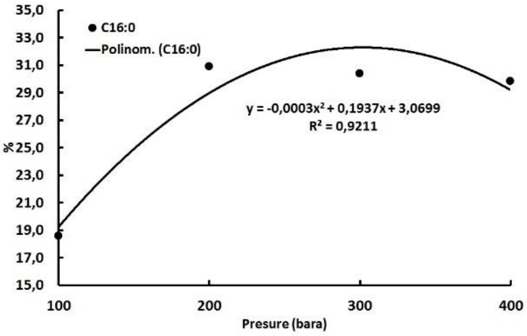


Figure 2. The relationship between pressure and C14:0 graph

Figure 3. The relationship between pressure and C15:0 graph



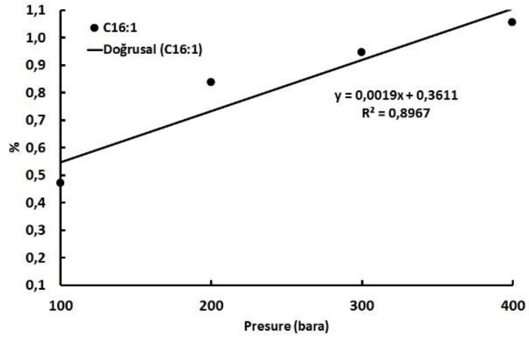
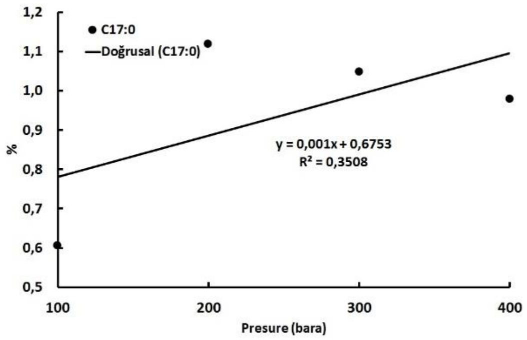


Figure 4. The relationship between pressure and C16:0 graph

Figure 5. The relationship between pressure and C16:1 graph



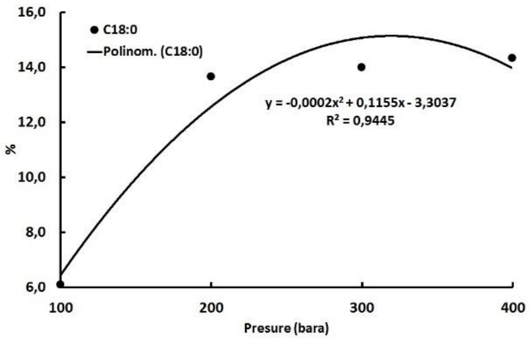
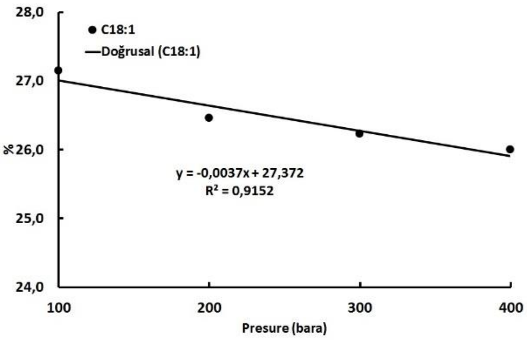


Figure 6. The relationship between pressure and C17:0 graph

Figure 7. The relationship between pressure and C18:0 graph



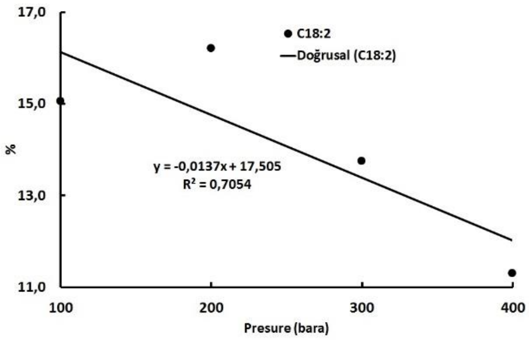
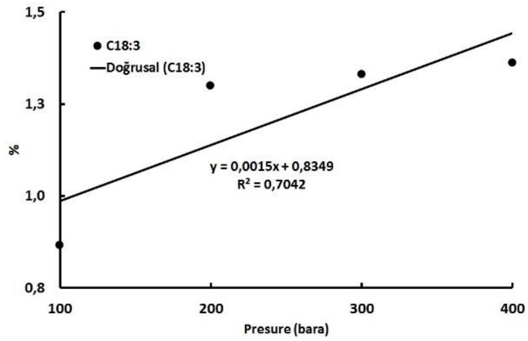


Figure 8. The relationship between pressure and C18:1 graph

Figure 9. The relationship between pressure and C18:2 graph



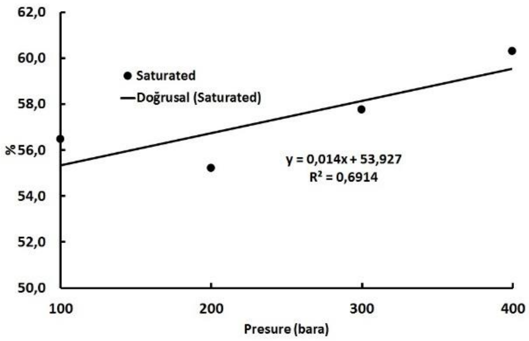


Figure 10. The relationship between pressure and C18:3 graph

Figure 11. The relationship between pressure and the saturated graph

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