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Experimental Study of *Dorema aucheri* Extraction with Supercritical Carbon Dioxide

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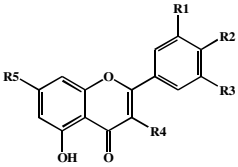
Dorema aucheri Boiss. is a native Iranian medicinal plant that is used as food. This plant is rich in flavonoids. Flavonoids are compounds found in fruits, vegetables and certain beverages that have diverse beneficial biochemical and antioxidant effects. This medicinal plant pose great benefits for human health. In this work the extraction of flavonoid compounds from this plant, with supercritical carbon dioxide, is studied. Using supercritical CO_2 seems to be a promising and alternative process because of operating at low temperature and acceptable mass transfer rate with no solvent residual at the last product. The operating conditions are pressure between 15 to 20 MPa and temperature between 313 to 323 K. After analyzing the extracted products by GC and GC/MS, α-eudesmol (39.2 %) and δ-cadinene (12.9 %) were the major bioactive flavonoid compounds in this plant. The optimum pressure, temperature and dynamic extraction time were also determined.

Key Words: *Dorema aucheri*, Medicinal plant, Supercritical extraction, Flavonoid, α-Eudesmol.

INTRODUCTION

In recent years there is an increasing interest to replace chemically synthesized compounds by natural equivalents that can be found mostly in plant materials. Iran is one of the countries, has the largest medicinal plants with traditional agriculture. *Dorema aucheri* Boiss. (Apieaceae) is an umbelliferae species in the flora of Iran, full of flavonoid compounds. Fig. 1 shows this medicinal plant and its flavonoid structure.



Flavonol	R1	R2
Quercetin	OH	H
Kaempferol	H	H
Myricetin	OH	OH
Isorhamnetin	Me	H

Fig. 1. *Dorema aucheri* plant , structure of this flavonoid , the type of flavonols

Flavonoids are a kind of highly effective antioxidant[1-3]. These compounds are a kind of highly effective natural antioxidant that control the blood cholesterol and triglyceride and used especially in kidney problems[4]. Although this plant is used more traditionally there is less study about its physiological property and the best way of its extraction. Supercritical fluid extraction (SFE) is a new and powerful developing technique in separation process that produces bioactive compounds[5]. The extraction of flavonoid compounds using supercritical fluids is important due to the high purity of the final compounds, which increases the added value of the final products and their price in the international market[6-8]. The supercritical fluid is usually CO_2 because is not toxic, no flammable, with low cost , operates under mild conditions ($P \geq 74$ bar and $T \geq 31$ °C), physiologically harmless, environmentally safe, non-explosive, and readily available and it can be easily removed from products. Nowadays, supercritical fluid extraction, is widely used as an attractive alternative extraction method to conventional liquid extraction in wide variety areas including the industries of food, pharmacy, environmental engineering, chemical and oil industries[9-12]. Optimization of the experimental conditions is a critical step in the development of a successful supercritical fluid extraction process due to the effect of various variables on the extraction efficiency [13,14]. There are three extraction modes with supercritical fluids: dynamic, static, and static-dynamic[15]. In this research the static-dynamic mode was used. Fig. 2 shows a profile of dynamic system. This profile can be divided into three distinct regions. The initial extraction of material in region I occurs rapidly and is dependent upon the solubility of the bulk analyte in the supercritical fluid. Region II is an intermediate region where the extraction process occurs at a slower rate of extraction due to diffusion controlled kinetics. Region III represents the portion of the extraction where the process is truly diffusion limited. The present work studied Iranian *Dorema aucheri* leaves, measuring the oil yield and the concentration of flavonoid extraction with supercritical carbon dioxide at

pressures: 150, 175, and 200 bar and temperatures of 40, 45 and 50 ºC. The objective was to find the effect of several supercritical fluid extraction parameters (pressure, temperature and dynamic extraction time) on the supercritical CO_2 extraction of flavonoid compounds from *Dorema aucheri* and the optimum operational conditions for the best yield. It is expected to call the attention of the industrial units in Iran where there are many plants with a great potential as solid matrices for obtaining useful compounds with applications in the food and pharmaceutical industries.

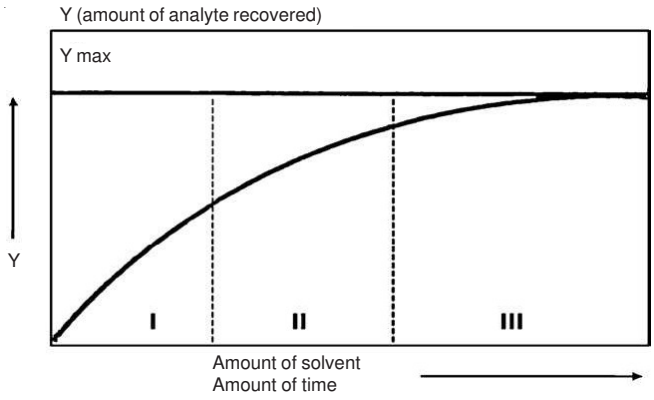


Fig. 2. Extraction yield *versus* time and mass of carbon dioxide[15]

EXPERIMENTAL

The plant was used in this study named *Dorema aucheri* (Apieaceae) with the herbarium No. of AR337E that was collected close to the city of Yasuj in Iran (30º, 19.644' Northern and 51º, 44.885' Eastern). Air dried *Dorema aucheri* was grounded in a blender to produce a fine powder with proper particle size. Dichloromethane with certified purity of 99.9 % was provided by Merck Co., Germany. The CO_2 used in this study with purity higher than 99.9 %, obtained from Eritch gas Co., Iran.

Characterization of the *Dorema aucheri* fixed bed: The fixed bed was formed with 5 g of *Dorema aucheri*, which have been milled in the desired size, added to the extractor in small portions. Care was taken to obtain a uniform bed avoiding wall effects and channeling.

Experimental procedure: The experiments were carried out in a bench scale apparatus. Carbon dioxide is feed from a tank gas and liquefied by a condenser, then pumped through a shell and tube form surge tank. So that warm water is circulated in its shell with constant temperature. Prior to the extraction, the material is usually grinded to increase the surface area in contact with the supercritical solvent and also increase the accessibility of the solute inside cell structures, thus increasing mass transfer kinetics. This enables the description of the resulting particles using the basic geometries of slab, cylinder or sphere. For this study, about 5 g of dried *Dorema aucheri* and glass beads were loaded in high pressure vessel with internal volume 250 mL. Glass beads prevent channeling of the flow in packed beds and the dead volume. The static time in this study is 45 min, then dynamic conditions started by interning the supercritical-CO_2 to the cell and opening the outlet back pressure valve. The product was collected in a U-tube separator. The outlet was immersed into the liquid dichloromethane and the temperature of the solvent was kept at below 0 ºC using ice and salt both during dynamic extraction time. Then CO_2 goes from a gas meter. Fig. 3 shows the schematic diagram of the extractor apparatus and Fig. 4 is the experimental system.

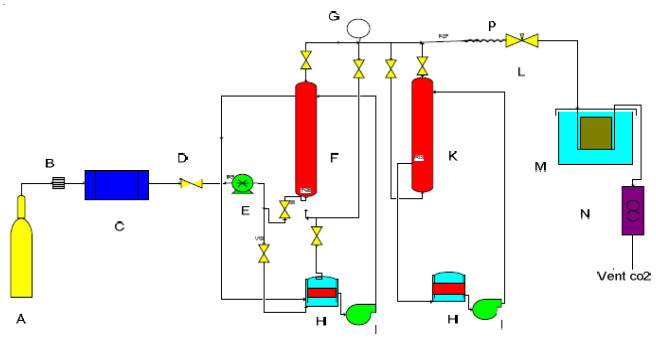


Fig. 3. Schematic diagram of the extraction of flavonoids from *Dorema aucheri* plant with supercritical carbon dioxide: A: CO_2 tank; C: Condenser; D: Check valve ; E: pump; F: Surg tank; G: Pressure gauge ; H: Warm baths ; I: Water pump ; K: Extraction column ; L: Restrictor valve; M: Sample collection vessel; N: Gas meter; P: Temperature controlled restrictor valve

Fig. 4. Laboratory extraction system by supercritical fluid technology

Experimental conditions: In order to find the conditions of supercritical extraction of *Dorema aucheri* oil that results in the higher flavonoid compounds concentration, the experimental design shown in Table-1 was performed. Each experiment had 45 min of static extraction and 90 min dynamic extraction with a CO_2 flow rate of 0.4 L/min. Each set of conditions was tested by duplicate for the appropriate data and measuring the maximum content of oil in the samples.

Flavonoids analysis: Analysis was performed according to reported procedures[16,17] using a Hewlett-Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60 ºC for 3 min, programmed to 220 ºC at a rate of 5 ºC/min and kept constant at 220 ºC for 5 min. The flow rate of helium as carrier gas was 1 mL/min. MS spectra were taken at 70 eV. The compounds were identified by comparison of RRI, DB5 with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra.

RESULTS AND DISCUSSION

It has been proven that the optimum extraction pressure and temperature for obtaining high yield of flavonoids resulting in negligible solubility of the other components are in the ranges from 180 to 200 bar and from 40 to 50 ºC[8,9]. The range of process parameters such as pressure and temperature are given in Table-1.

TABLE-1
EXPERIMENTAL DESIGN FOR *Dorema aucheri* LEAVES OIL EXTRACTION WITH SUPERCRITICAL CO_2

Temperature (K)	Pressure (MPa)
313	15.0
	17.6
	20.0
318	15.0
	17.5
	20.0
323	15.0
	17.5
	20.0

The yield of flavonoids by experiments are presented in Figs. 5 and 6. In these figures, sharp variation of the yield is observed at initial period of extraction because at the static condition, some flavonoids are extracted and gathered at the outer surface of the particles. State solutes in the outer parts of particles are extracted much faster than the solutes in the inner parts of particles at the beginning of the dynamic extraction. As the extraction time proceeds, the diffusion of solutes from inner parts to the bulk phase becomes more difficult due to the decrease in driving force between the solid and fluid phases leading to the reduction of extraction rate.

Experimental data: The results obtained for the extraction of *Dorema aucheri* with supercritical CO_2 are plotted in Figs. 5 and 6. Table-2 depicts, the oil of *D. aucheri* at each temperature and pressure, consists of eight monoterpene hydrocarbons about (5.3 %), 3 oxygenated monoterpenes (4 %), 15 sesquiterpene hydrocarbons (37.0 %), 6 oxygenated sesquiterpenes (35.6 %) and 3 aliphatic compounds (7.3 %). α-eudesmol (39.2 %) and δ-cadinene (12.9 %) were the major compounds in this oil.

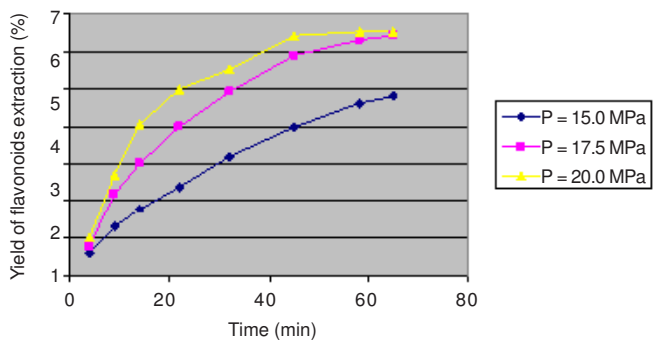


Fig. 5. Effect of pressure on the extraction yield at T = 318 K

Effect of pressure: It is observed that increasing pressure improves the yield, up to 20 MPa. Such a change of Y *versus* pressure is due to dual effect. On one hand, adding the pressure causes higher supercritical-CO_2 density, therefore improves the solubility of flavonoids and leads to higher Y. On the other hand, increasing pressure reduces the supercritical-CO_2 diffusivity then resulting to lower Y.

Effect of temperature: Fig. 6 shows the effect of temperature on Y at pressure of 20 MPa. Like the pressure, temperature displays a nonlinear and complex effect on the extraction near or above the critical point. Increasing the temperature, on one hand, decreases the supercritical-CO_2 density. While higher temperature (323 K) lowers down the yield. Fig. 6 shows that the inverse effect of temperature begins around 323 K in this process.

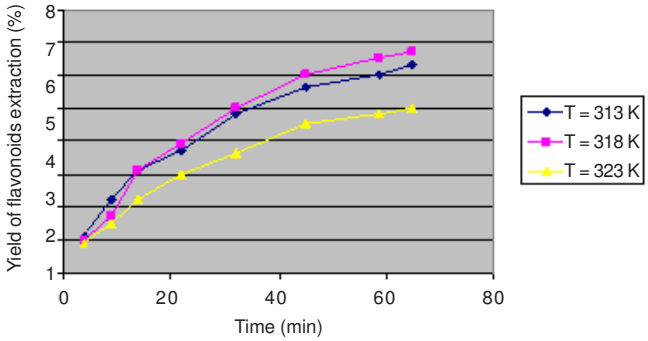


Fig. 6. Effect of temperature on the extraction yield at P = 20 MPa

Effect of dynamic extraction time: Figs. 5 and 6 show the effect of dynamic time on the extraction yield of *Dorema aucheri* leaves in supercritical-CO_2 with applying several different pressures and temperatures. At each figure, the extraction yield is increased with dynamic time until 90 min. It can be concluded that the solvent power of supercritical CO_2 density is reduced due to the lower CO_2 density and maximum yield was obtained at 90 min. However, at higher pressures (20 MPa) the extraction rate is higher and as a consequence the extraction yield kept increasing but after 60 min of extraction time, the extraction yield dropped. Thus, the highest extraction yield was achieved at 60 min dynamic time (Fig. 5).

Conclusion

The combination of P = 20 MPa and T = 318 K, provided the best yield (65.79 %) among the tested conditions and the greatest effect on the extraction yield. Selectivity of the extraction was observed from temperature, then pressure and dynamic extraction time. The concentration of key compounds (α-eudesmol and δ-cadinene) do not decrease with the increment of temperature and pressure in the tested range. It was shown that *Dorema aucheri* leaves is a potential source of flavonoid compounds. α-Eudesmol (39.2 %) and δ-cadinene (12.9 %) were the major determined bioactive flavonoid compounds. High recovery obtained by using supercritical carbon dioxide extraction. So supercritical fluid technology is now considered to be an innovative and promising way to design and modify pharmaceutical substances. This method allows for easy removal of the solvent by depressurization and control of extraction condition by variation of temperature, pressure or time.

TABLE-2
PERCENTAGE COMPOSITION OF THE *Dorema aucheri* SAMPLES EXTRACTED WITH SUPERCRITICAL-CO_2 AT DIFFERENT CONDITIONS

Extraction conditions (MPa/K)	15/313	17.5/313	20/313	20/318	17.5/318	15/318	15/323	17.5/323	20/323
α-Pinene	0.63	0.68	0.76	0.8	0.82	0.74	0.68	0.68	0.77
Camphene	0.12	0.16	0.15	0.2	0.2	0.17	0.15	0.14	0.18
β-Pinene	0.14	0.17	0.17	0.2	0.18	0.17	0.16	0.15	0.16
Myrcene	0.18	0.21	0.31	0.3	0.28	0.26	0.25	0.26	0.27
Limonene	0.4	0.9	1.2	1.4	1.32	1.28	1.24	1.18	1.21
(E)β-Ocimene	0.3	0.44	0.56	0.6	0.51	0.4	0.38	0.36	0.42
γ-Terpinene	0.11	0.17	0.23	0.3	0.28	0.27	0.26	0.25	0.28
Terpinolene	0.7	0.9	0.9	1	0.87	0.76	0.65	0.53	0.64
Thymol-methyl ether	0.12	0.11	0.22	0.2	0.19	0.21	0.28	0.11	0.14
Bornyl acetate	0.14	0.06	0.14	0.2	0.18	0.17	0.18	0.2	0.2
Methyl geranate	1.7	1.9	2.8	3.6	3.1	3.21	3.43	3.21	3.48
α-Cubebene	0.07	0.06	0.08	0.1	0.08	0.07	0.064	0.091	0.1
α-Copaene	1	1.3	1.9	2.1	1.5	1.6	1.76	1.97	2.09
β-Patchoulene	0.11	0.2	0.28	0.3	0.27	0.19	0.21	0.28	0.29
β-Caryophyllene	1.2	2.5	3.7	3.9	3.1	3.2	3.4	3.6	3.6
α-Santalene	1.2	1.34	1.67	1.5	1.12	1.23	1.43	1.49	1.5
β-Gurjunene	1.12	1.3	2	2.2	1.9	2	2.1	2.3	2.48
Aromadendrene	0.21	0.3	0.48	0.5	0.34	0.4	0.43	0.46	0.5
α-Humulene	0.16	0.12	0.17	0.2	0.17	0.18	0.186	0.19	0.12
Germacrene D	0.45	0.67	0.76	0.8	0.73	0.68	0.54	0.3	0.76
β-Selinene	2	2.2	2.2	2.3	2.1	2	1.97	1.8	1.9
Viridiflorene	2.8	3.2	3.27	3.3	3.15	3.1	2.9	2.67	2.8
γ-Cadinene	11.7	11.9	12.76	12.9	11.18	11.1	11	10.87	11.2
δ-Cadinene	1.7	1.87	2.23	2.2	2.1	2	1.8	1.67	2
Cadina-1,4-diene	0.13	0.18	0.18	0.2	0.16	0.15	0.21	0.19	0.2
Germacrene B	2.9	2.87	3	3.3	3.1	3.3	3.4	3	2.9
Germacrene D-4-ol	0.18	0.25	0.29	0.3	0.27	0.26	0.2	0.29	0.27
trans-Sesquisabinene hydrate	0.48	0.53	0.67	0.6	0.5	0.46	0.39	0.44	0.57
Hexadecan	1.78	1.9	2	1.2	1	1.1	1.14	1.19	1.22
2-Pentadecanone	2.9	3.1	3.45	3.9	3.2	3.4	3.1	2.96	3.1
γ-Eudesmol	0.18	0.19	0.19	0.2	0.16	0.15	0.14	0.13	0.12
Cubenol	0.46	0.54	0.59	0.6	0.54	0.63	0.48	0.43	0.44
α-Eudesmol	37.12	38.4	39	39.2	38.12	37	36.5	39.1	39
α-Cadinol	1.92	2.02	2.1	2.2	2	2.12	2	2.12	2.4
Hexadecanoic acid	0.16	0.18	0.21	0.2	0.18	0.16	0.2	0.23	0.19

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