Supercritical Fluid Extraction of *Euphorbia rigida*

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1 Introduction

The steadily increasing consumption of crude oil and apparent insecurity in the supply and price of foreign petroleum during the late 1970's have boosted research on the substitution of traditional hydrocarbon fuels by alternative renewable resources for feedstock chemicals [1].

Euphorbia rigida, a member of the Euphorbiaceae family, is one such renewable source. Not suited for food production it grows abundantly in arid and semi-arid regions in western and south-western Anatolia, Turkey [2–4]. This family of plants includes roughly 2000 species, ranging from small herbs to large trees. The majority of them can produce a milky latex that yields wide range of chemical such as rubber, oils, terpenes, waxes, hydrocarbons, starch, resins, tannins, and balsams of interest to various industries. In recent years *Euphorbia* species have become attractive as petro-crops because of their hydrocarbon potential. Several species of *Euphorbia* such as *Euphorbia lathyris* and *Euphorbia tirucalli* are being cultivated to evaluate their potential as biosources of chemical feedstock [2, 5, 6].

Soxhlet extraction and pyrolytic conversion are the traditional methods for extraction of bio-oil and natural products from the plant material, but the former is labor intensive and time consuming, requires large volumes of solvents, and pollutes the environment [7–9]. Pyrolytic techniques are equally undesirable due to the high temperatures used, also entailing loss of essential oils. With the demand for more environmentally friendly methods and increased productivity, supercritical fluid extraction (SFE) has been evaluated. This method offers several advantages, such as low viscosities, high diffusivities and fast mass transfer, leading to rapid extraction. Although many supercritical fluids have been used in SFE, the most popular one is CO_2 because of its easily attainable supercritical condition at 75.3 atm and 31 °C. Carbon dioxide is also readily available in a highly pure state. It is inexpensive, non-polar, non-toxic, chemically inert, and is able to solvate a wide range of organic compounds including those having higher molecular mass. However, the limitation of CO_2 is that the polar organic compounds are often difficult to extract from plant materials though they are soluble in supercritical CO_2. The extraction of polar molecules requires addition of a modifier, most commonly methanol [10–13].

In this study, supercritical CO_2 was used to extract aliphatic hydrocarbons with recoveries comparable to those of other conventional extraction techniques [13–15]. After SFE of *Euphorbia rigida*, quantitative analysis of the hydrocarbons was performed by gas chromatography. Finally, GC/MS analysis was undertaken to confirm the identity of the hydrocarbons and some of the polar compounds such as aldehydes, alcohols, esters, *etc*.

2 Experimental

2.1 Sample

Euphorbia rigida was collected from south-western Anatolia between Afyon and Denizli. The plants were harvested between April to June, dried, and stored in a cool and a dark room for six months. The plants' leaves and stalks were ground in a blender to produce a fine powder.

2.2 Extraction Procedure

Supercritical fluid extractions were performed using SFE grade CO_2 and an Isco Model 100 D syringe pump operated at 400 atm; CO_2 was cooled to −10 °C and −5 °C (Julabo F10 cooler). The plant material was mixed with (1:1) glass beads (Alltech Associates, 100 μm o.d. silanized) prior to loading into an extraction cell (2.2 mL volume cell from Keystone Scientific). The cell was placed in an extractor (Isco SFX-2.10) which consists of a temperature controller, a vent valve, an on/off valve, an extraction cell, and another on/off valve to maintain the extraction cell at the required temperature. The extractor was connected with the restrictor via a finger-tight union (Keystone). The flow rate of the supercritical fluid through the extraction cell was measured as liquid CO_2 at the pump and was controlled by 10 cm long restrictors (30 μm i.d.) cut from fused-silica tubing. Extracted analytes were collected in a 21 mL collection vial with a screw cap (with a hole) and PTFE-laminated silicon septa. Methylene chloride (5 mL) was used as a trapping solvent.

The plant material was sequentially extracted with pure CO_2 (at 400 atm, 50 °C) for 30 min, followed by CO_2 + 10% CH_3OH (*v/v*) (at 400 atm, 50 °C) first in the static mode for 15 min to accomplish equilibrium in the cell and subsequently in the dynamic mode for 30 min. Fractions were collected at

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set time intervals for both the pure CO_2 and CH_3OH-modified CO_2 extractions. The accuracy of the temperature and the pressure measurements (of the full scale of the pump) were ±1 °C and ±2%, respectively. Measurements were carried out in triplicate with a standard deviation of less than 0.6%.

After SFE, the sample (residue) was removed from the extraction cell and placed in a vial and sonicated with 10 mL of CH_2Cl_2 for 4 h. The solvent was then evaporated to 1.5 mL and C_{19}-nonadecane was added as internal standard to the sample for GC-FID analysis. The SFE recoveries from *Euphorbia rigida* were then compared with the hydrocarbon recoveries obtained by the Soxhlet process.

To determine 100% recovery, the plant material (1 g) was placed in a cellulose thimble, transferred to a Soxhlet extractor and extracted for 8 h with 75 mL CH_2Cl_2. A vacuum evaporator was then used for evaporation of the solvent at 30 °C. The SFE and Soxhlet extracts were fractionated in a silica-gel column with pentane to recover the hydrocarbon fraction. First, silica-gel (Fisons, 30–70 mesh) was dried at 170 °C and then placed in a chromatographic column (45 cm × 1.6 cm i.d.). Samples were loaded onto the column and eluted with 75 mL pentane. Fractions were collected in a 100 mL flask and pentane was evaporated off at 30 °C under vacuum.

UV spectra (Unicam UV2-100) were recorded in CH_3OH by using 1 cm^3 cell. An appropriate wavelength range (190 nm–900 nm) was chosen to analyze the extracts.

GC-FID analysis was performed (Hewlett-Packard 5890) with helium as carrier gas on a BP1 capillary column (25 m × 0.32 mm i.d.; 0.5 µm film thickness) from SGE (Scientific Glass Engineering). The injection port and detector were both heated at 310 °C. The GC oven was ramped from 40 °C (2 min hold) at 15 K min^{-1} to 300 °C. The splitless injection mode was used. Quantitative determination of the hydrocarbons was based on comparison of peak areas with those of the internal standard. An *n*-alkane standard from C_{14} to C_{40} (Aldrich) (2.91 mg each) was prepared in pentane (10 mL) and stored in a refrigerator at 5 °C.

GC/MS analysis was carried out on a Carlo Erba model HRGC 5160 and a VG-Trio 1000 (Fisons) mass spectrometer. A BPX5 capillary column (SGE; 25 m × 0.32 mm i.d.; 0.5 µm film thickness) was used. The mass spectrometer was set to scan between *m/z* 40 and 400, total ion current (TIC) and selective ion monitoring (SIM) modes; the electron-impact ionizing voltage was 70 eV.

3 Results and Discussion

3.1 Fractionation

The yield of Soxhlet extraction of 1 g of *Euphorbia rigida* 86.4 mg g^{-1} and that of SFE was 74.9 mg g^{-1}. These were further fractionated using a silica-gel column with pentane as eluent. As can be seen from **Table 1**, the percentage of extractable hydrocarbons obtained by Soxhlet extraction (0.19%) was lower than by SFE (0.21%). The yield of hydrocarbons after column fractionation from SFE was 0.27 wt %, *i.e.* 30% more hydrocarbons than the Soxhlet (0.19%) extract. Thus, supercritical CO_2 extracted the majority of the hydrocarbons. The modifier was used to recover more polar high molecular weight hydrocarbons.

Table 1. The percentages of extract by SFE and Soxhlet and the percentages of hydrocarbons in SFE and Soxhlet.

Extraction Type	Conditions	Extractable plant material (wt %)	Extractable hydrocarbons from plant (wt %)
SFE	CO_2/50 °C	4.02 ± 0.60[a]	0.21 ± 0.04[a]
	CO_2 + 10% CH_3OH/50 °C	1.99 ± 0.13[a]	0.06 ± 0.003[a]
	Residue[b]	1.48 ± 0.22[a]	–
Soxhlet	8 h/CH_2Cl_2	8.64 ± 0.039[a]	0.19 ± 0.02[a]

[a] Values in ± are the standard deviation of triplicate extractions.
[b] Sonication of plant residue in CH_2Cl_2 for 4 h.

Not all of the extractable material consisted of hydrocarbons; some pigments such as taraxanthin (413 nm), lycopene (456 nm), and chlorophyll (a) (665 nm) are also extracted, as determined by UV spectroscopy [16].

3.2 GC-FID of SFE and Soxhlet Extracts

The hydrocarbons pre-fractionated with pentane using a silica-gel column were determined by GC with the aid of external and internal standards (**Table 2**, **Figure 1**, **Figure 2**).

Table 2. Comparison of CO_2, CO_2 + 10% CH_3OH, sonication in CH_2Cl_2, and Soxhlet the recovery of the quantitation of the hydrocarbons from *Euphorbia rigida*.

Species	Concentration (µg g^{-1})		
	CO_2[a]	CO_2+10% CH_3OH[b]	Soxhlet[c]
C_{13}	5.8	ND	ND
C_{15}	2.3	ND	ND
C_{20}	8.7	ND	9.9
C_{21}	3.9	ND	2.6
C_{22}	ND	ND	11.0
C_{23}	16.2	ND	35.9
C_{24}	ND	ND	32.8
C_{25}	23.9	4.6	31.9
C_{26}	3.6	ND	7.3
C_{27}	11.7	3.1	24.4
C_{28}	32.7	ND	6.4
C_{29}	672	10.4	486
C_{30}	2.3	ND	ND
C_{31}	73.4	8.4	88.1
C_{33}	26.3	2.1	27.1

See **Figure 1** and **Figure 2** for chromatographic results. ND = not detected.
[a] Sample extracted at 400 atm, 50 °C CO_2 for 30 min.
[b] Sample extracted at 400 atm, 50 °C CO_2 + 10% CH_3OH for 30 min.
[c] Sample extracted with Soxhlet apparatus, 8 h, in CH_2Cl_2.

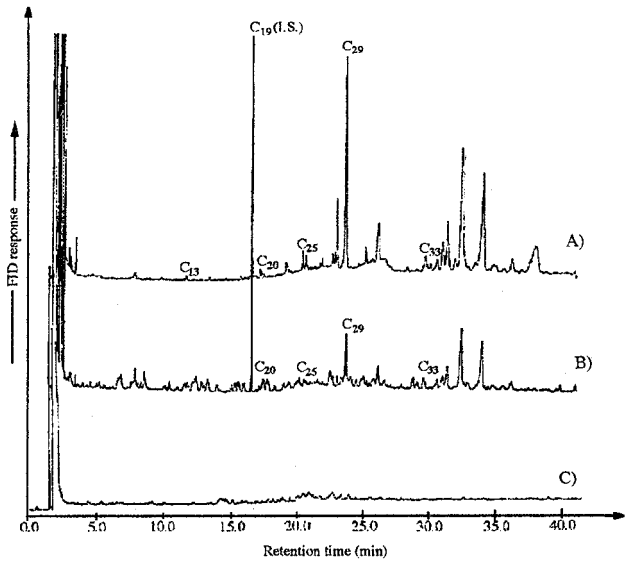

Figure 1. GC analysis of SFE sample from *Euphorbia rigida* on a BP1 capillary column. A) 50 °C, 400 atm CO_2 B) 50 °C, 400 atm CO_2 + 10% CH_3OH C) Sonication in CH_2Cl_2.

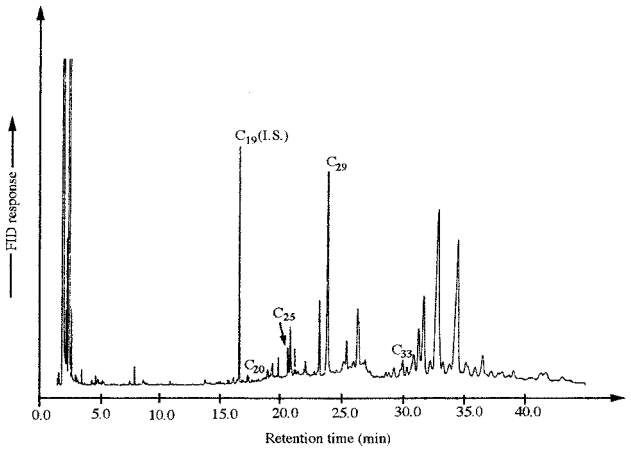

Figure 2. GC analysis of a Soxhlet extract from *Euphorbia rigida* on a BP1 capillary column.

The recovery of hydrocarbons from the SFE-CO_2 extract (0.88 mg g^{-1}) is higher than that of SFE-CO_2 + CH_3OH modifier (0.029 mg g^{-1}) and of Soxhlet extraction (0.76 mg g^{-1}).

3.3 GC/MS of SFE-CO_2 Extract

The SFE extracts analyzed by GC/MS (**Figure 3**). Although the total ion current chromatogram is quite complex and contains several overlapping peaks (including a large hump at 22–24 min), the reconstructed selected ion current chromatograms for alkanes (m/e = 57) and alkenes (m/e = 55) show clearly resolved chromatographic peaks that could be used for quantification of the individual species [17]. This extract consists of some alkanes, alkenes, and a free fatty acid, alcohols, an ester, an aldehyde, and some tetracyclic triterpenoid compounds (**Table 3**).

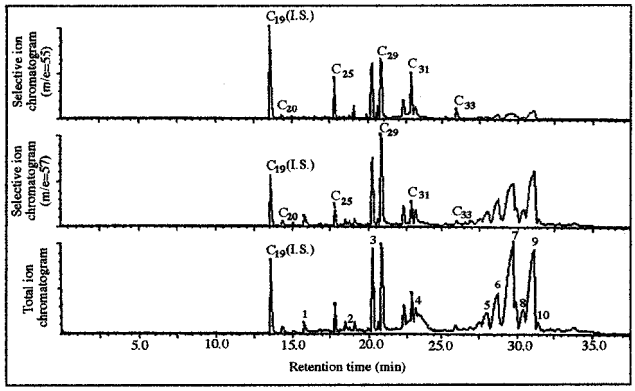

Figure 3. Total ion and selected ion GC/MS chromatograms of extract of *Euphorbia rigida* on a BPX5 capillary column.

Table 3. The identification of some tetracyclic triterpenoid compounds by using GC/MS (Numbers refer to **Figure 3**).

Numbers	Name of compounds
1	9-Octadecenal
2	1-Eicosanol
3	Heneicosyl formate
4	1-Heptacosanol
5	Kauren-18-ol-acetate, (4β)
6	Cholest-8-en-3-ol, 14-methyl-, (3β, 5α)-
7	9,19-Cyclolanostan-3-ol, 24-methylene, (3β)-
8	9,19-Cyclo-9β-lanostane-3β, 25-diol
9	Ergost-25-ene-3,5,6,12-tetrol, (3β, 5α, 6β, 12β)
10	9,19-Cyclolanost-23-ene-3,25-diol-3-acetate, (3β, 23-E)

4 Conclusions

SFE has been shown to successfully extract hydrocarbons from *Euphorbia rigida*. SFE was complete within 60 min, which is eight times faster than Soxhlet extraction. This method was easier to perform and inexpensive; moreover, the consumption of solvent (1–2 mL) was lower than in Soxhlet extraction (75 mL).

The per-sample cost of SFE grade CO_2 is often only 1 or 2% of equivalent extraction solvents. For instance, the SFE extraction of a 1 g sample at an average CO_2 flow rate of 0.21 mL min^{-1} and 30 min time requires approximately 0.63 g of CO_2. This cost is less than US Dollar 0.01 as compared to US Dollar 5–20 for comparable Soxhlet extraction solvents. A significant amount of electric energy can be saved as well. The implementation of SFE can eliminate long, high temperature reflux periods and solvent concentration evaporation steps. Furthermore, laboratory venting costs can be reduced.

Since supercritical fluids possesses low viscosity, high diffusivity and hence fast mass transfer is achieved, this leads to rapid extraction than Soxhlet extraction. The use of CO_2 as a

supercritical extraction fluid also reduces hazards to the environment.

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