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Comparison of supercritical fluid and Soxhlet extractions for the quantification of hydrocarbons from *Euphorbia macroclada*

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Abstract

This study compares conventional Soxhlet extraction and analytical scale supercritical fluid extraction (SFE) for their yields in extracting of hydrocarbons from arid-land plant *Euphorbia macroclada*. The plant material was firstly sequentially extracted with supercritical carbon dioxide, modified with 10% methanol (v/v) in the optimum conditions that is a pressure of 400 atm and a temperature of 50 °C and then it was sonicated in methylene chloride for an additional 4 h. *E. macroclada* was secondly extracted by using a Soxhlet apparatus at 30 °C for 8 h in methylene chloride. The validated SFE was then compared to the extraction yield of *E. macroclada* with a Soxhlet extraction by using the Student's *t*-test at the 95% confidence level. All of extracts were fractionated with silica-gel in a glass column to get better hydrocarbon yields. Thus, the highest hydrocarbons yield from *E. macroclada* was achieved with SFE (5.8%) when it compared with Soxhlet extractions (1.1%). Gas chromatography (GC) analysis was performed to determine the quantitative hydrocarbons from plant material. The greatest quantitative hydrocarbon recovery from GC was obtained by supercritical carbon dioxide extract (0.6 mg g^{-1}).

Keywords: SFE; Extraction; *t*-test; Hydrocarbons; Quantification

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1. Introduction

The use of large amounts of highly purified organic hazardous solvents, with high costs of both purchase and disposal, and its use over an extended period can create health problems. In addition long extraction times are needed, due to slow analyte diffusion and desorption from the sample matrix to the extraction fluid. Soxhlet generates dirty extracts, and the sample preparation has been estimated to constitute about two-thirds of the total time of analysis as well. As a consequence, there has been an increasing demand for new better and more environmentally friendly extraction methods in analytical chemistry [1–11]. Thus, supercritical fluid extraction (SFE) has received significant attention as an analytical tool for the extraction of hydrocarbons from such as plant materials [12–14], soils [15–17], and coal [18].

Carbon dioxide is the most common supercritical fluid used for analytical-SFE because of its favorable temperature–pressure critical points (31 °C; 75 atm), chemical inertness, availability, low cost, and safety. Furthermore, an extraction CO_2 can be depressurized to a gas at atmospheric pressure, thus leaving behind the extracted material. One of the more important advantages of using carbon dioxide for SFE is that, removal of extraction solvent and waste disposal are neither a major problem nor expense. The low polarity of CO_2, however, makes it an inappropriate extraction solvent for polar compounds. In order to enhance the solvating power of CO_2, the addition of a few percent of a modifier solvent, most commonly, methanol is required [11,19–23].

Since the oil crisis of the early 1970s, declining reserves and instable prices of fossil fuels have boosted the research for an alternative renewable hydrocarbon resource plants to replace petroleum all over the world. One of the renewable hydrocarbon resource plants is *Euphorbia macroclada* is a member of the Euphorbiaceae family and it grows abundantly in arid and semi-arid-land regions in Anatolia, Turkey. This family of plants includes roughly 2000 species, ranging from small herbs to large trees. The most of them can produce milky latex, which yields wide range of chemical such as rubber, oils, terpenes, waxes, hydrocarbons, starch,

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resins, tannins, and balsams of interest to various industries but not use for food production. In last 25 years, *Euphorbia* species have become attractive as petro-crops due to their hydrocarbon potential [12,24–27]. There is not found any information about the chemical composition of *E. macroclada* in the literature and this plant was chosen for this respect.

The objective of this study was to investigate the extraction of hydrocarbons from *E. macroclada* by using supercritical fluid extraction with the yield comparable to that of conventional Soxhlet extraction technique. Then, SFE and Soxhlet extraction of *E. macroclada*, quantitative analysis of the hydrocarbons was performed by gas chromatography (GC). At the end of the characterization, GC/MS analysis was undertaken to confirm the identity of hydrocarbons. In addition to, the validated SFE was then compared to the extraction yield of *E. macroclada* with a Soxhlet extraction.

2. Experimental

2.1. Materials

E. macroclada was collected from near Afyon, Turkey. The plant was harvested between April and June, dried and stored in a cool and dark room for 6 months. The plants' leaves and stalks were ground in a mill to produce a fine powder.

2.2. Extraction procedures

2.2.1. SFE

Supercritical fluid extractions were operated using pure CO_2 (99.9%) and an Isco Model 260 D syringe pump with a cooling jacket cooled by a 9505 Model circulating bath (Polyscience, USA) set at $-5\,^{\circ}C$. An amount of 1 g of plant material was mixed with 1 g of prewashed sea sand and placed into the extraction cell (10.4 ml, Keystone Scientific, Bellefonte, PA, USA) and the rest of the cell was then filled with sand. The extraction cell was placed inside a Carlo Erba GC oven. 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 restrictor (30 μm i.d.) cut from stainless steel tubing (Coopers Needle Works Ltd., Birmingham, UK). The restrictor was heated to prevent it from plugging. Extracted analytes were collected by placing the outlet of the restrictor into a 21 ml vial containing 5 ml of methylene chloride.

The plant material was extracted with CO_2 at pressures of 200 and 400 atm and at temperatures of 50 and 100 °C. The optimum yield was obtained at 400 atm and 50 °C and these parameters was then used for later experiments. For this respect, *E. macroclada* 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. Extracts were collected at set time intervals for both the pure CO_2 and CO_2–CH_3OH modified. The accuracy of the temperature and the pressure measurements (of the full scale of the pump) were $\pm 1\,^{\circ}C$ and $\pm 2\%$, respectively. Measurements were carried out in triplicate with a relative standard deviation of less than 9.6%.

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_{12}-dodecane was added as internal standard to the sample for GC–flame ionization detection (FID) analysis. The SFE recoveries from *E. macroclada* were then compared with the hydrocarbon recoveries obtained by the Soxhlet extraction.

2.2.2. Soxhlet

The plant material (1 g) was placed in a cellulose thimble, transferred to a Soxhlet extractor. The round bottom extraction flask was filled with 75 ml of methylene chloride, and extraction at the boiling point was allowed to proceed for 8 h. A vacuum evaporator was then used for evaporation of the solvent at 30 °C.

2.3. Fractionation procedure

The SFE and Soxhlet extracts were fractionated in a silica-gel column with *n*-pentane to recover the hydrocarbon fractions. First, silica-gel (60–120 Mesh) was dried at 170 °C and then placed in a chromatographic column (40 cm × 1.5 cm i.d.). Samples were loaded onto the column and eluted with 75 ml *n*-pentane. Fractions were collected in a 100 ml flask and *n*-pentane was evaporated off at 30 °C under vacuum.

2.3.1. Chromatographic analysis

GC–flame ionization detection (FID) were performed using a Hewlett-Packard 5890 gas chromatography with helium as carrier gas on a 25 m BP1 capillary column (SGE, 0.32 mm i.d.; 0.5 μm film thickness). Injections were performed in the splitless mode. The oven temperature program was 40 °C (2 min hold) followed by a $15\,^{\circ}C\,min^{-1}$ temperature ramp to 300 °C followed by a 20 min hold. Quantitative determination of the hydrocarbons was based on comparison of peak areas with those of the internal standard. An *n*-alkane standard from C_{12} to C_{40} (Aldrich) was prepared in *n*-pentane (10 ml) and stored in a refrigerator at 4 °C.

GC/MS analysis was carried out on GCQ Finnigan Mat Model GC and ion-trap-mass spectrometer. A CP-Sil 8 CBMS capillary column (25 m × 0.32 mm i.d.; 0.5 μm film thickness) was used. The mass spectrometer was set to scan between *m*/*z* 20 and 350, total ion current (TIC), and selective ion monitoring (SIM) modes, the electron-impact ionizing voltage was 70 eV.

3. Results and discussion

3.1. *Effect of system temperature and pressure of SFE*

At the constant pressure of 400 atm, when the extraction temperature was elevated from 50 to 100 °C, extraction yield dropped from 5.1 ± 9.6% to 2.8 ± 11.5% indicating that higher extraction yield was achieved at lower extraction temperature. This result was not surprising since the solvent strength of a supercritical fluid usually increases with its density [4]. Therefore, elevated temperatures would result in a decreased density of supercritical carbon dioxide. Thus, an extraction temperature of 50 °C was chosen for later experiments because of increased the yield of extract from plant material.

At the constant temperature of 50 °C, when the extraction pressure was increased from 200 to 400 atm, the yield rate of extracts increased from 2.20 ± 10.8% to 5.1 ± 9.6% indicating that higher extraction pressures resulted in higher the yield of the extract. The results were expected because above the critical point, increasing the pressure at a constant temperature will increase the density of supercritical CO_2 and increase the solvating power of the supercritical fluid [28]. Moreover, the dielectric constant of CO_2 increases with increasing pressure in the supercritical phase. Thus, higher pressure would have more extracting capability. Because a better extraction yield of extracts was achieved at the extraction pressure of 400 atm, it was chosen for later experiments.

3.2. *Comparison of supercritical fluid and Soxhlet extractions*

After the optimum (temperature and pressure) extraction conditions of the SFE were determined, this procedure was compared with the Soxhlet extraction, a conventional method. SFE were also performed in triplicate and show a relative standard deviation (R.S.D.) of about 9.6% which are significantly higher than R.S.D. for five times Soxhlet extractions (6.5%). The results are shown in Table 1. The extraction yield of the supercritical fluid and the Soxhlet extractions were evaluated statistically using the Student's *t*-test at the 95% confidence level [29–31]. There were no significant differences between these two extraction methods in the extraction yield (5.1% versus 5.7% extract, respectively, for SFE and Soxhlet extraction) and is attributed to random error. Thus, the Soxhlet extraction can offer comparative extraction yield with supercritical fluid extraction. The variances of these two extraction methods evaluated using the *F*-test at the 95% confidence level indicated that there were no significant differences (2.35×10^{-5} versus 1.37×10^{-5}, respectively, for SFE and Soxhlet extraction method). As a result, the Soxhlet extraction method gives higher extract yields and good repeatability than that of supercritical fluid extraction.

Table 1
Comparison of the yield of extracts with SFE and Soxhlet extraction

Extraction type	Number of extraction (*n*)	Extract (wt.%)	R.S.D. (%)[a]
SFE	3	5.1	9.6
Soxhlet	3	5.7	6.5

[a] R.S.D.: relative standard deviation.

Table 2
The yield of hydrocarbons from SFE and Soxhlet extraction

Extraction type	Number of fraction (*n*)	Hydrocarbon yield (wt.%)	R.S.D.[a]
SFE	3	5.8	6.0
Soxhlet	3	1.1	18.6

[a] R.S.D.: relative standard deviation.

3.3. *Chromatographic separation and detection*

The extracts that were obtained from SFE and Soxhlet extractions were fractionated using a silica-gel column with *n*-pentane as eluent and the results were given in Table 2.

The percentage R.S.D. value of hydrocarbon from Soxhlet extract (18.6%) was much larger than that of SFE (6.0%).

Table 3
Comparison concentration ($\mu g\, g^{-1}$) of hydrocarbons in *E. macroclada* obtained from CO_2, $CO_2 + 10\%\ CH_3OH$ and Soxhlet extractions

Species	Concentration ($\mu g\, g^{-1}$)		
	CO_2[a]	$CO_2 + 10\%\ CH_3OH$[b]	Soxhlet[c]
C_{13}	–	1.4 ± 0.1[d]	–
C_{14}	–	1.4 ± 0.1[d]	2.5 ± 0.1[d]
C_{15}	–	0.7 ± 0.1[d]	8.8 ± 0.1[d]
C_{16}	18.8 ± 0.1[d]	21.7 ± 0.2[d]	1.9 ± 0.1[d]
C_{17}	1.4 ± 0.2[d]	–	–
C_{18}	13.0 ± 0.1[d]	10.1 ± 0.1[d]	4.3 ± 0.1[d]
C_{19}	5.8 ± 0.1[d]	2.9 ± 0.1[d]	9.4 ± 0.1[d]
C_{20}	59.2 ± 0.1[d]	53.4 ± 0.1[d]	11.4 ± 0.1[d]
C_{21}	2.9 ± 0.1[d]	5.8 ± 0.1[d]	3.7 ± 0.1[d]
C_{22}	49.1 ± 0.2[d]	15.9 ± 0.1[d]	5.2 ± 0.1[d]
C_{23}	4.3 ± 0.1[d]	–	6.0 ± 0.1[d]
C_{24}	7.2 ± 0.1[d]	2.9 ± 0.1[d]	5.5 ± 0.1[d]
C_{25}	14.4 ± 0.2[d]	1.4 ± 0.1[d]	18.4 ± 0.1[d]
C_{26}	180.4 ± 0.1[d]	176.1 ± 0.2[d]	15.8 ± 0.1[d]
C_{27}	7.2 ± 0.1[d]	1.4 ± 0.1[d]	11.8 ± 0.1[d]
C_{28}	64.9 ± 0.1[d]	10.1 ± 0.1[d]	17.9 ± 0.1[d]
C_{29}	5.8 ± 0.1[d]	8.7 ± 0.1[d]	3.3 ± 0.1[d]
C_{30}	26.0 ± 0.1[d]	11.6 ± 0.1[d]	3.1 ± 0.1[d]
C_{31}	13.0 ± 0.1[d]	2.2 ± 0.1[d]	16.5 ± 0.1[d]
C_{32}	1.4 ± 0.1[d]	–	8.7 ± 0.1[d]
C_{33}	–	–	–
C_{34}	20.2 ± 0.1[d]	–	0.3 ± 0.1[d]
C_{35}	57.7 ± 0.1[d]	–	0.3 ± 0.1[d]
C_{36}	–	–	0.1 ± 0.1[d]

[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, 30 °C with 75 ml CH_2Cl_2 for 8 h.

[d] Values in ± are the standard deviation of triplicate injection.

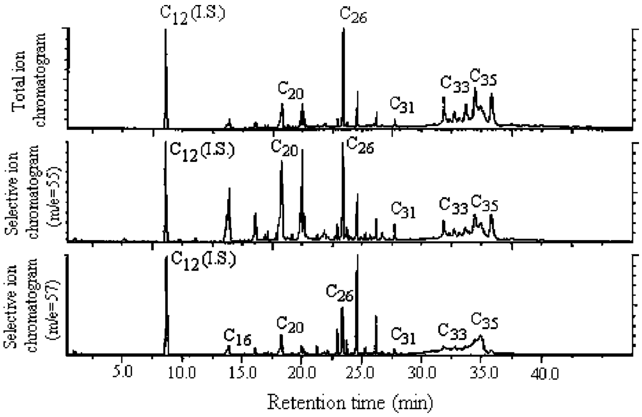

Fig. 1. Total ion and selected ion GC/MS chromatograms of extract of *E. macroclada* on a CP-Sil 8 CBMS capillary column.

The high R.S.D. for hydrocarbon from Soxhlet is due to the small concentration of hydrocarbons. The yield of hydrocarbons after column fractionation from SFE was 5.81 wt.%, i.e. 82% more hydrocarbons than that of Soxhlet (1.1 wt.%) extract. Thus, supercritical CO_2 extracted the majority of the hydrocarbons and SFE showed excellent precision (6.0 versus 18.6). It is much more selective than Soxhlet hydrocarbon fraction.

The hydrocarbons obtained from column fractionation of extracts were quantitatively determined by GC with aid of external and internal standards (Table 3). The yield of hydrocarbons from the SFE-CO_2 extract ($0.6\,mg\,g^{-1}$) is higher than that of SFE-$CO_2 + CH_3OH$ modifier ($0.3\,mg\,g^{-1}$) and of Soxhlet extraction ($0.2\,mg\,g^{-1}$). This result is well agreement with fractionation results. As shown in Table 3, the distributions of hydrocarbons, which are in a maximum range of C_{20}–C_{31} and in a minimum range of C_{13}–C_{20} were obtained for *E. macroclada*.

The SFE extracts, which is including the highest amount of hydrocarbons, were analyzed by GC/MS (Fig. 1). Analysis of extracts by GC/MS showed that, in most cases, the total ion chromatogram (TIC) contained many chromatographic background was such that peaks due to hydrocarbons could not be detected (resolved) at all. It was possible to confirm by monitoring selected ions ($m/e = 57$, for alkanes and $m/e = 55$, for alkenes) for the hydrocarbons of interest.

4. Conclusions

This study demonstrated that SFE is suitable for the routine extraction of hydrocarbons from plant material. The most striking advantages of analytical-SFE over conventional Soxhlet extraction were in the selectivity of the extraction, much cleaner extracts (see Fig. 1), less time (1 h instead of 8 h), and less hazardous organic solvent (5 ml instead of 75 ml).

Although the extraction yields obtained by conventional Soxhlet extraction, were higher than those of obtained by supercritical fluid extraction and also showed higher accuracy (5.7% versus 5.1%) and precision (6.5 versus 9.6) than supercritical fluid extraction, after column fractionation of these extracts, SFE extract showed higher hydrocarbon yield (5.8% versus 1.1%) than Soxhlet extract and SFE method was also exhibited excellent precision (6.0 versus 18.6) than Soxhlet extraction method.

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