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RESEARCH ARTICLE

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# Green extraction of oil from *Carum carvi* seeds using bio-based solvent and supercritical fluid: Evaluation of its antioxidant and anti-inflammatory activities

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## Abstract

**Introduction:** The consumption of health-promoting products such as oil seeds may improve human health and prevent certain diseases. Carvi seeds have the potential to produce oil with nutritional and functional properties rich in active compounds.

**Objective:** To extract bioactive lipids from *Carum carvi* seeds using green methodologies.

**Material and methods:** Supercritical-carbon dioxide (Sc-$CO_2$) and ethanol as co-solvent and bio-based solvent 2-methyltetrahydrofuran (MeTHF) were used to extract the oil from *Carum carvi*. The yield, the chemical composition, as well as antioxidant and anti-inflammatory activities of green extracted oils were investigated and compared to those obtained with conventional methods (hexane and Folch system).

**Results:** MeTHF extraction gave higher oil yield than that obtained by hexane. Fatty acids composition of the two obtained green extracted oils was similar to conventional extracted ones where petroselinic (39–43%), linoleic (29–31%) and oleic (19–21%) acids were the major compounds. Furthermore, MeTHF and Sc-$CO_2$ green extracted oils were enriched of bioactive compounds including sterols (5.4 and 7.3 mg/g oil) and total polyphenols (9.3 and 7.6 mg GAE/g oil) which were correlated to enhanced antiradical capacity. Moreover, the green extracted oils exhibited high anti-inflammatory capacity inhibiting nitric oxide (NO) release in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages with $IC_{50}$ values of 28 and 24 μg/mL.

**Conclusion:** Green solvents are a good alternative to petroleum solvents to recover oil from carvi seeds with high amount of nutritionally important fatty acids, along with significant antioxidant and anti-inflammatory potential.

KEYWORDS

bioactivities, carvi seeds, green extraction, MeTHF, oil, supercritical fluid

  

## 1 | INTRODUCTION

Oilseeds are of great interest because of their high nutritive, functional as well as industrial values. They can provide diet with a high concentration of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) which are beneficial for human health and can protect against cardiovascular diseases.[1] Oils contain minor compounds with important biological properties such as phytosterols and polyphenols. Sterols are powerful antioxidants and effective cholesterol-lowering compounds and some dietary sterols such as β-sitosterol may protect against several cancers.[2] Furthermore, phenolic components are potent antioxidants and exhibit beneficial effect on plasma lipoproteins, and lipid oxidation. Polyphenols exert also a potent effect on inflammation process and oil phenolics are now being increasingly recognised for their anti-inflammatory and antioxidant effects on the skin.[3]

Recently, besides oleaginous species, several new plants have been investigated as new sources of functional vegetable oils. Studies have stated that seeds of some Apiaceae species are sources of valuable oil in particular carvi (*Carum carvi*).[4] The different plant parts of carvi including fruits, roots and shoots are commonly used as condiment for flavouring food and beverages.[5] The oil obtained from the seeds is used for the flavouring of ice cream, candy, pickles, and soft drinks.[6] The seeds are used traditionally as a remedy to treat various illnesses such as bloating, diarrhoea, dyspepsia, flatulence, morning sickness, colic, and as spasmolytic. They are also used in bronchopulmonary disorders and as a cough remedy, as well as an analgesic.[6-8] *Carum carvi* seeds contain a significant amount of oil with high concentration of the MUFAs; petroselinic and oleic acids. After oxidation, petroselinic acid can yield lauric and adipic acids which are used in the synthesis of detergents and nylon polymers. This fatty acid is also used in cosmetic formulations as a hydrating, anti-ageing, and skin-irritation reducing agent.[9]

Conventionally, oils are obtained using mechanical process or solvent extraction. The mechanical method often affords low yields, while chemical extraction methods such as Soxhlet, Folch or Bligh and Dyer use hazardous organic solvents.[10] Hexane, a paraffinic petroleum fraction, is one of the most used solvent to extract oil because of high oil yield recovery.[11] However, hexane is listed as an hazardous air pollutant by the US Environmental Protection Agency (EPA) and it has been reported by the EPA Toxic Release Inventory that more than 20000 t of hexane are released to the atmosphere each year from the extraction of vegetable oils.[12] In addition, exposure to hexane leads to neurotoxic damage.[13] Therefore, considering such toxicological and environmental concerns, there is a need to find safe alternative to *n*-hexane without compromising oil recovery.[14]

The concept of green chemistry is governed by principles which are mainly aimed to reduce wastes and to promote a more efficient use of energy and resources. Regarding the green extraction of natural products, six specific principles have been proposed including: (i) innovation by selection of varieties and use of renewable plant resources, (ii) use of green solvents and principally water or agro-solvents, (iii) reduction of energy consumption by energy recovery and using innovative technologies, (iv) production of co-products instead of waste to include the bio- and agro-refining industry, (v) reduce unit operations and favour safe, robust and controlled processes, and (vi) aim for a non-denatured and biodegradable extract without contaminants.[15] Supercritical fluid extraction (SFE) is an attractive clean alternative to organic solvents and an environmentally friendly technology for food and pharmaceutical product extraction using green solvents.[16] Carbon dioxide ($CO_2$) is the most commonly used supercritical fluid due to lack of toxicity and flammability and low cost.[17] Some studies have reported on the good yields of lipids extraction from plant material using supercritical fluids.[18,19] However, bio-based solvents constitute an interesting new green alternative to petrochemical solvents.[15] Indeed, these solvents are produced from biomasses such as starch, corn, citrus, and soybeans and have a high solvent power and are biodegradable, low toxicity, low flammability and cost effective.[15,20] In this context, 2-methyltetrahydrofuran (MeTHF) is a green solvent derived from lignocellulosic biomass.[21,22] It has chemical and physical properties similar to hexane but has no carcinogenic, no mutagenic and no toxic properties.[23] Moreover, it is biodegradable.[21,22,24] Recently, bio-based solvents including MeTHF, limonene and *p*-cymene have been found to be effective to extract lipids from several plant seeds.[11,25,26] Some green methods including ultrasonic assisted extraction have been applied to extract volatiles compounds from carvi.[27,28] However, to our knowledge there are no previous reports on chemical content of carvi oil obtained with green extraction procedures. As a continuation of efforts in developing healthy oils rich in health beneficial components, the present study was designed to investigate the performance of supercritical-$CO_2$ (Sc-$CO_2$) and MeTHF compared to conventional extraction techniques for extraction of oil from *C. carvi* seeds.

## 2 | EXPERIMENTAL

### 2.1 | Reagents and plant material

Solvents including methanol (99.8% purity), *n*-hexane (98% purity), chloroform (99% purity) were purchased from Loba Chimie (Mumbia, India). The bio-based solvent MeTHF (≥ 99% purity) was supplied by Sigma-Aldrich (Taufkirchen, Germany). Betulin, 2,7 dichlorofluorescein, pyridine, *N*,*O*-bis(trimethylsilyl)trifluoracetamide with trimethylchlorosilane, sodium carbonate ($Na_2CO_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, dimethyl sulphoxide (DMSO), gallic acid, Folin Ciocalteu's phenol reagent and Nω-nitro--L-arginine methyl ester hydrochloride (L-NAME) were supplied by Sigma-Aldrich. Fatty acid methyl esters (FAME) mix was supplied by Supelco (Bellefonte, PA, USA). RPMI-1640 medium, fetal bovine serum (FBS) and penicillin/streptomycin were from PAN-Biotech (Aidenbach, Germany).

*Carum carvi* (Apiaceae family) seeds were purchased from Korba (northeast of Tunisia) on July 2016. Plant identification was carried out by Professor Abderrzek Smaoui (Biotechnology Centre in Borj-Cedria, Tunisia). A voucher specimen (BC2011–2026) was deposited at the herbarium of the Laboratory of Aromatic and Medicinal Plants,

Biotechnology Centre in Borj Cedria. The seeds were ground into a fine powder using a grinder just before extraction.

## 2.2 | Extraction procedures

### 2.2.1 | Soxhlet extraction

Thirty grams of seeds were transferred to a cellulose thimble and put into the extraction chamber of a Soxhlet apparatus fitted with a condenser. The latter was placed on a distillation flask filled with 250 mL of *n*-hexane or MeTHF. Samples were extracted under reflux for 6 h. After the extraction, the solvents were evaporated using a rotary evaporator. The extracted oils were weighed, closed under a nitrogen stream and stored at −20°C until further analysis.

### 2.2.2 | Chloroform/methanol extraction

Twenty grams of ground seeds were mixed with a solution of chloroform/methanol in a 2:1 *v*/*v* ratio and mixed thoroughly in a vortex according to Folch *et al*.[29] Then, the mixture was filtered through Whatman paper filter into a separatory funnel and a potassium chloride solution (80 mL) was added. After gentle shaking, the mixture was allowed to separate into two layers. The lower layer was evaporated under reduced pressure. The oil sample was weighed and flushed with nitrogen, and stored at −20°C until further analysis.

### 2.2.3 | Supercritical carbon dioxide (Sc-$CO_2$) extraction

Oil was extracted from carvi seeds with Sc-$CO_2$ using pilot-scale equipment (Separex, Champigneulles, France). The extracted oil was maintained at 20 MPa pressure and 40°C temperature.[30] The extraction time was fixed to 180 min under a continuous flux of $CO_2$ (14 mL/min). After finishing the extraction processes, total extraction yield was measured. Obtained oil was transferred into glass bottles, flushed with nitrogen and stored at −20°C until analysis.

## 2.3 | Fatty acid analysis

Fatty acid composition of the extracted oils was determined using gas chromatography–flame ionisation detection (GC-FID). First, the fatty acids were transformed into their corresponding methyl esters (FAME) using sodium methoxide solution.[31] The FAME were analysed by GC using HP 6890 gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with RT-2560 capillary column (100 m length, 0.25 mm i.d., 0.20 μm film thickness). Nitrogen was used as carrier gas. The initial oven temperature was held at 170°C for 2 min, increased at a rate of 3°C/min ramp to 240°C and finally held there for 15 min. The injector and detector temperatures were 225°C. Individual fatty acids were identified by comparing their retention times with a certified FAME mix.

## 2.4 | Sterol analysis

Oil samples were saponified with 2 M potassium hydroxide (KOH) in ethanol, together with 1 mg betulin as internal standard. The solution was heated for 1 h at 70°C. After saponification, the mixture was washed by water and the unsaponifiables were extracted using diethyl ether. The ether phase was washed with water, dried over anhydrous sodium sulphate, and the solvent was removed under vacuum and nitrogen. The unsaponifiable fraction was charged on a basic silica thin-layer chromatography (TLC) plate and sprayed with a 2,7 dichlorofluorescein ethanolic solution. Sterols bands were identified under ultraviolet (UV) light, scraped off with a spatula, then extracted with chloroform and evaporated to dryness.

Sterols were silylated by the addition of pyridine and *N*,*O*-bis(trimethylsilyl)trifluoracetamide containing 1% trimethylchlorosilane solution and heated at 90°C for 30 min. Identification and quantification of sterols as trimethylsilyl ethers was carried out on an Agilent Technologies 6890 GC–MS. An Agilent Technologies HP 5 MS column (0.25 μm × 250 mm × 30 m) was used. The splitless injector was at 275°C, and the temperature programme was programmed to rise from 50 to 275°C at a rate of 30°C/min. The ionisation potential was fixed at 70 eV. Scan time and mass range were 1 s and 30–450 (*m*/*z*), respectively. Sterols were identified by comparing their retention times with those of commercially available standards and results were expressed as milligrams per gram (mg/g) of oil.

## 2.5 | Total polyphenols content

The phenolic compounds were extracted from oils according to the method of Parry *et al*.[32] Briefly, 0.5 g of oil extracted with 2.5 mL of methanol. The sample was vortexed and then centrifuged at 5000 rpm for 5 min and the supernatant was collected. This procedure was repeated two more times. All three extractions were combined and the resulting phenolic solution was then lyophilised and stored at −20°C until analysis.

Total phenolic content of the seed oils were determined using Folin–Ciocalteu reagent. Briefly, an aliquot of the phenolic extract was added to distilled water and mixed for 30 s. Then, Folin–Ciocalteu reagent (125 μL) was added and allowed to react for 3 min. Afterward $Na_2CO_3$ solution was added. After incubation in the dark for 2 h, the absorbance versus prepared blank was read at 760 nm. Gallic acid was used as standard and results were expressed as milligram of gallic acid equivalent per gram (mg GAE/g) of oil.

## 2.6 | Measurement of antioxidant activity

The radical scavenging capacity of carvi seed oil was determined using DPPH radicals as described by Parry *et al*.[32] slightly modified method. Briefly, freshly made 0.2 mM DPPH-methanol solution was mixed into seed oil extract at different concentrations. The vigorously mixed mixture was placed in darkness for 30 min. The absorbance was measured at 517 nm against blank using a UV-visible spectrophotometer. The

antiradical activity was expressed as half maximal inhibition concentration ($IC_{50}$) (in μg/mL). All samples were analysed in triplicate.

## 2.7 | Measurement of anti-inflammatory activity

### 2.7.1 | Cell culture

RAW 264.7 murine macrophage cells were supplied from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cell line was cultured in RPMI 1640 medium supplemented with 10% (*v*/*v*) FBS, 100 U/mL of penicillin, 100 μg/mL of streptomycin and were maintained at 37°C in humidified atmosphere of 5% $CO_2$.

### 2.7.2 | Measurement of nitrite production

RAW 264.7 cells were seeded in 24-well plates at a density of $2 \times 10^5$ cells per well and were allowed to attach for 24 h at 37°C and given a fresh change of medium. Cells were then treated with various concentrations of carvi oils dissolved in the DMSO or the positive control (L-NAME). The final concentration of solvent in the culture medium was maintained at 0.1% (*v*/*v*) to avoid solvent toxicity. Cells were then stimulated with 100 μg/mL lipopolysaccharide (LPS) and incubated at 37°C under 5% $CO_2$. After 24 h, cell-free supernatants were collected and nitrite production was assessed using Griess method.[33] The absorbance at 540 nm was then measured using an automated 96-well Varioskan Ascent plate reader and nitric oxide (NO) levels, produced by murine macrophage-like RAW 264.7 cells, was determined by comparison with a sodium nitrite ($NaNO_2$) standard curve.

## 2.8 | Statistical analysis

Data for analytical methods were reported as the mean ± standard deviation (SD). Duncan test ($P < 0.05$) was performed to determine significant differences among means of experiments carried out in triplicate. For cell experiments, data are reported as standard error of the mean (SEM) and values of at least three independent experiments are given. An analysis of variance (ANOVA) with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. The *P*-values less than 0.01 were considered statistically significant.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Oil extraction yield

*Carum carvi* seeds were extracted using new technologies including a green solvent (MeTHF) and a green method (Sc-$CO_2$ extraction) and compared to conventional methods including extractions with hexane in soxhlet apparatus for 6 h and Folch method.

According to Figure 1, carvi seed oils had 13 and 18% yields based on conventional methods including extraction by hexane and Folch method, respectively. Such differences could be due to the effect of the extraction method. *n*-Hexane was reported to be less effective than Folch's procedure regarding the oil extraction from coriander, almonds and hazelnuts seeds.[34]

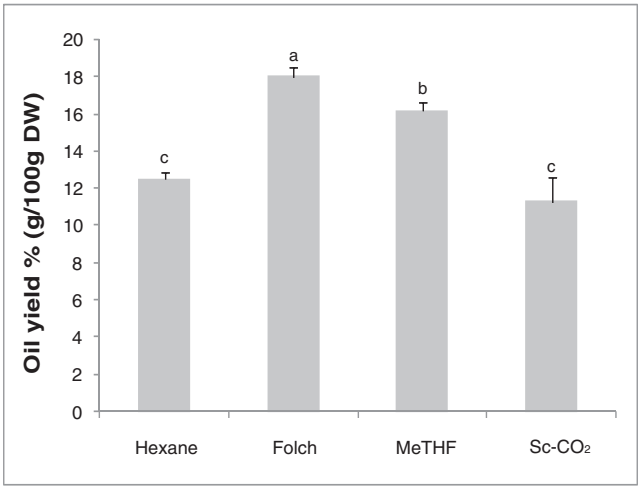


**FIGURE 1** Oil yield of *Carum carvi* seeds obtained with conventional (Soxhlet and Folch) and green (MeTHF and Sc-$CO_2$) extractions. Different letters on the top of data bars indicate significant differences ($P < 0.05$) between mean values (± SD, $n = 3$). DW, dry weight; MeTHF, 2-methyltetrahydrofuran; Sc-$CO_2$, supercritical carbon dioxide; SD, standard deviation

As is shown in Figure 1, green techniques recovered oil with relatively good yields. Interestingly, extraction by bio-based solvent MeTHF recovered a high yield (16%) which was superior to the one obtained by hexane (13%) and close to the one obtained by Folch method. These results showed that MeTHF is a suitable solvent to extract oil from carvi seeds. Such efficiency could be due to its apolar aprotic chemical characteristics.[35] MeTHF has been reported to exhibit the ability to dissolve triacylgylcerols and phospholipids in rapeseed oil giving similar yield to the one obtained by hexane.[36] Ben Youssef *et al*.,[25] extracted date palm seeds of three cultivars (Deglet Nour, Allig, and Belah) with the two solvents (hexane and MeTHF) for 8 h by soxhlet and reported a cultivar-dependent effect where MeTHF was the best solvent to extract oil from Bellah and Allig varieties while hexane remained the better solvent to extract oil from Deglet Nour variety. Moreover, MeTHF appeared to be a good candidate to replace hexane for lipid extraction from oleaginous yeast biomass.[37]

However, there was no significant difference between *n*-hexane and Sc-$CO_2$ extraction using temperature 40°C, pressure 200 bar and ethanol as entrainer (Figure 1). In agreement with our study, high pressure (200–300 bar) and low temperature (40°C) were used for oils extraction using Sc-$CO_2$ from rosehip[38] and rice bran[19] seeds. High pressures are recommended to increase the density and, consequently, the solvating power of solvent, which increases the solubility of oil in $CO_2$ and thus the yield.[19,39] Moreover, Sc-$CO_2$ is a valuable green technology which allows the production of oil without residues – "solvent free extracts" which is of interest to the food industry.

Overall, these experimental data show that green techniques especially the bio-based solvent MeTHF represent a suitable alternative solvent for lipids extraction from carvi seeds.

## 3.2 | Fatty acid composition

The fatty acid composition of the *C. carvi* seed oils obtained from conventional and green extractions is summarised in Table 1. The content of unsaturated fatty acids (UFAs) was high in carvi oils extracted with hexane and Folch method accounting for 93 and 92%, respectively. MUFAs was the most represented subclass with percentages of 62 and 63%, respectively, followed by PUFAs with percentage of 30% in the two oils. These values are similar to those obtained by Kozłowska *et al.*[40] who reported MUFA level of 63% in carvi oil extracted by hexane and chloroform/methanol. However, Laribi *et al.*[41] showed lower MUFA contents in hexane oil extracted from different carvi ecotypes (50–56%).

Our results (Table 1) demonstrated that carvi oil extracted with green methods exhibited chemical composition comparable to the ones obtained with conventional methods. In fact, UFAs accounted for 93% in MeTHF and Sc-$CO_2$ oils. Green extraction resulted in oils rich in MUFAs (61 and 63%) and PUFAs (32 and 30%) for MeTHF and Sc-$CO_2$ oils, respectively, while, the level of saturated fatty acids (SFAs) was very low (7% in both oils). SFE technique can provide low temperatures of extraction and so a high product quality.[42]

Overall, the results proved that green techniques are as able as hexane and chloroform/methanol to extract oil rich MUFAs and PUFAs. These have beneficial effects on both normal health and chronic diseases, such as regulation of lipid level, cardiovascular and immune functions.[43]

As shown in Table 1, petroselinic acid (C18:1 n-12) was the major fatty acid in conventional extracted oils with high percentages reaching 43% in hexane and 40% in Folch extracted oils, followed by linoleic acid (30% in the two oils) and oleic acid (19 and 22% in hexane and Folch extracted oils). Our results are consistent with previous studies indicating that petroselinic acid is the main fatty acid in carvi oil after hexane and chloroform/methanol extractions with percentages varying from 29 to 41% of total fatty acids according to seeds origin.[4,40]

Fatty acid composition of green extracted oils exhibited a similar trend to that of the conventional ones (Table 1). Indeed, petroselinic acid (C18:1 n-12) remained the major compound with comparable levels of 40 and 43% in MeTHF and Sc-$CO_2$ extracted oils, respectively. Moreover, linoleic and oleic acids were the second and the third most representative fatty acids with percentages of 32 and 21%, respectively in MeTHF oil and 30 and 21%, respectively in Sc-$CO_2$ oil. Some studies investigated the effect of green extraction on the chemical composition of petroselinic acid-containing oils. MeTHF was reported to have the same ability compared to hexane to extract the main fatty acids, petroselinic and oleic, from rapeseed.[36] Likewise, Ben Youssef *et al.*[25] study emphasised that there were no significant changes in fatty acid profiles of the date oils obtained by MeTHF and hexane. Although Sc-$CO_2$ extraction affected coriander oil yield, there was no significant changes in the fatty acid profile between hexane and Sc-$CO_2$ extracted oil.[44]

According to our results, it can be concluded that green extraction methods can be considered interesting alternative technologies to conventional methods to obtain carvi oil rich in fatty acids with health and economic-enhancing value especially petroselinic acid.

## 3.3 | Sterol content

The main constituents of carvi unsaponifiables are sterols and polyphenols.[40] As it can be shown in Table 2, total sterols in conventional carvi extracted oils were 6.45 and 4.5 mg/g dry weight (DW) after hexane and Folch extractions, respectively. Kozłowska et al.[40] reported slightly higher total sterols content (about 7 mg/g DW) in carvi oil extracted by hexane and chloroform/methanol.

Our results showed that green extraction of carvi oil sterols using MeTHF gives oil with comparable content (5.3 mg/g DW) to

**TABLE 1** Chemical composition (%) of *Carum carvi* oils obtained with conventional (Soxhlet and Folch) and green [2-methyltetrahydrofuran (MeTHF) and supercritical-carbon dioxide (Sc-$CO_2$)] extractions

| Fatty acid composition (%) | Hexane | Folch | MeTHF | Sc-$CO_2$ |
|---|---|---|---|---|
| C16:0 | 5.08 ± 0.12b | 6.90 ± 0.22a | 5.25 ± 0.16b | 5.16 ± 0.01b |
| C16:1 | Tr | Tr | 0.10 ± 0.01a | 0.18 ± 0.01a |
| C18:0 | 1.66 ± 0.03ab | 1.73 ± 0.15a | 1.69 ± 0.08a | 1.58 ± 0.08b |
| C18:1 n-12 | 43.41 ± 4.53a | 39.21 ± 0.23b | 40.32 ± 2.68b | 43.52 ± 0.48a |
| C18:1 n-9 | 19.41 ± 0.86c | 21.28 ± 0.57a | 20.47 ± 1.20b | 19.02 ± 0.77c |
| C18:2 n-6 | 29.82 ± 3.61c | 30.39 ± 0.69b | 31.54 ± 1.75a | 29.62 ± 0.46c |
| C18:3 n-3 | 0.09 ± 0.04d | 0.48 ± 0.04b | 0.62 ± 0.04a | 0.35 ± 0.05c |
| C20:0 | 0.54 ± 0.12a | — | — | 0.54 ± 0.03a |
| ∑SFA | 7.27 ± 0.02b | 8.63 ± 0.07a | 6.94 ± 0.24c | 7.27 ± 0.11b |
| ∑UFA | 92.74 ± 0.01a | 91.37 ± 0.07b | 92.95 ± 0.59a | 92.55 ± 0.12a |
| ∑MUFA | 62.82 ± 3.67a | 60.51 ± 0.79b | 60.79 ± 1.48b | 62.54 ± 0.29a |
| ∑PUFA | 29.92 ± 3.66b | 30.88 ± 0.73b | 32.16 ± 1.83a | 30.01 ± 0.41b |

Values are means ± standard deviation. Different superscript letters (a–d) in a row indicate significant differences for individual fatty acid ($P < 0.05$). Abbreviations are: Tr, trace; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

**TABLE 2** Sterol content in *Carum carvi* oils obtained with conventional (Soxhlet and Folch) and green [2-methyltetrahydrofuran (MeTHF) and supercritical-carbon dioxide (Sc-$CO_2$)] extractions

| Sterols composition (mg/g oil) | Hexane | Folch | MeTHF | Sc-$CO_2$ |
|---|---|---|---|---|
| Cholesterol | 0.23 ± 0.01a | 0.14 ± 0.01c | 0.15 ± 0.01b | 0.22 ± 0.02ab |
| Cholesterol (7) | 0.06 ± 0.00a | 0.03 ± 0.00c | 0.04 ± 0.00b | 0.06 ± 0.01a |
| Campesterol | 0.36 ± 0.01b | 0.29 ± 0.01d | 0.31 ± 0.01c | 0.40 ± 0.02a |
| Stigmasterol | 1.79 ± 0.09b | 1.43 ± 0.06d | 1.51 ± 0.09c | 2.04 ± 0.12a |
| $^{7}$-Campesterol | 0.03 ± 0.00a | 0.02 ± 0.00b | 0.02 ± 0.00b | 0.03 ± 0.00a |
| β-Sitosterol | 3.21 ± 0.07b | 2.41 ± 0.11d | 2.57 ± 0.15c | 3.61 ± 0.20a |
| Sitostanol | 0.03 ± 0.0d | 0.05 ± 0.00b | 0.04 ± 0.01c | 0.06 ± 0.01a |
| $^{5}$-Avenasterol | 0.41 ± 0.03b | 0.09 ± 0.01c | 0.40 ± 0.05b | 0.48 ± 0.03a |
| $^{7}$-Avenasterol | 0.34 ± 0.01c | 0.06 ± 0.00d | 0.35 ± 0.06b | 0.36 ± 0.06a |
| Total sterols | 6.45 ± 0.21b | 4.53 ± 0.19d | 5.38 ± 0.38c | 7.26 ± 0.43a |

Values are means ± standard deviation. Different superscript letters (a–d) in a row indicate significant differences for individual sterols ($P < 0.05$).

conventional extractions, however, carvi oil was slightly enriched in total sterols (7.25 mg/g DW) after Sc-$CO_2$ extraction. It has been reported that the extractivity of phytosterols by Sc-$CO_2$ is facilitated by the presence of solvent such as ethanol and acetone in the solvent stream.[45] Moreover, Sicaire *et al.*[36] reported that rapeseed oil extracted with MeTHF was equivalent to oil extracted with *n*-hexane in total sterol content.

However, the conventional extracted oils were characterised by the presence of β-sitosterol (3.2 and 2.45 mg/g DW, respectively, in hexane and Folch) and stigmasterol (1.8 and 1.45 mg/g DW, respectively, in hexane and Folch) as major sterols. Cholestrol, campsterol and avensterols were present with low content also. This is in accordance with previous studies showing that carvi oil is a source of β-sitosterol and stigmasterol.[40] This tendency was found in green extracted oils; β-sitosterol and stigmasterol were the major compounds (Table 2). However, Sc-$CO_2$ extracted oil contained slightly higher amounts of β-sitosterol (3.6 mg/g DW) and stigmasterol (2 mg/g DW) than MeTHF extracted oil (β-sitosterol and stigmasterol contents of 2.5 and 1.5 mg/g DW, respectively). Sicaire *et al.*[36] found that the major sterols were identified in the same proportions in hexane and MeTHF rapeseed extracted oils. Recently, Prache *et al.*[46] showed that chloroform used for the separation of lipid classes such as non-polar sterols might be replaced efficaciously by several bio-based solvents including MeTHF. Furthermore, Sajfrtová *et al.*[47] found that the Sc-$CO_2$ extraction achieved at a pressure of 15 MPa and 40°C temperature, was a convenient extraction technique for obtaining a sea buckthorn extract concentrated with β-sitosterol.

Sterols are of great interest as high value-added products because of their nutraceutical activities. Overall, our results showed that green procedures are as effective as hexane and chloroform/methanol for the extraction of sterols from carvi seeds.

## 3.4 | Total polyphenols content and antioxidant capacity

Among bioactive compounds, phenolics constitute an important class since they may act as antioxidants and may have the potential to protect biologically important components, such as membrane lipids, DNA and proteins, from radical-mediated oxidative damage.

Total phenolic content (TPC) of carvi oils was evaluated. As shown in Table 3, conventional oils displayed high phenolic content with 5.6 and 6.6 mg GAE/g oil in hexane and chloroform/methanol oils, respectively. The TPC was higher than that reported by Kozłowska *et al.* [40] (in the oil of caraway seeds from Poland extracted by hexane and chloroform/methanol (0.07 and 0.78 mg GAE/g oil, respectively). Such differences could be explained by the effect of origin. Indeed, location and environmental conditions have been reported to significantly modulate phenolic compounds biosynthesis and accumulation in plant organs and oils.[48] However, similar TPC was reported by Ramadan[49] in oils from Apiaceae seeds such cumin (3.9 mg GAE/g oil) and coriander (4.3 mg GAE/g oil).

The results indicated that carvi green extracted oils were enriched of phenolic compounds compared to conventional ones (Table 3). Indeed, MeTHF and Sc-$CO_2$ oils displayed TPC of 9.1 and 7.3 mg GAE/g oil,

**TABLE 3** Total polyphenols contents and antioxidant activity of *Carum carvi* oils obtained with conventional (Soxhlet and Folch) and green [2-methyltetrahydrofuran (MeTHF) and supercritical-carbon dioxide (Sc-$CO_2$)] extractions

| | Hexane | Folch | MeTHF | Sc-$CO_2$ | BHT |
|---|---|---|---|---|---|
| Total phenolics (mg GAE/g oil) | 5.95 ± 0.32d | 6.61 ± 0.26c | 9.31 ± 0.65a | 7.25 ± 0.18b | — |
| DPPH radical activity ($IC_{50}$ μg/mL) | 247.50 ± 2.5b | 519.67 ± 6.81a | 116.67 ± 5.77c | 101.67 ± 2.89d | 11.5 ± 0.02 |

Values are means ± standard deviation. Different superscript letters (a–d) in a row indicate significant differences ($P < 0.05$). BHT, butylated hydroxytoluene; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

respectively, which were 1.6- and 1.3-folds higher than hexane extracted one. Ethanol has been reported to aid the SFE of polar phenolic compounds from canola press cake as compared to *n*-hexane extraction.[50] The use of polar co-solvent such as ethanol may enhance the solvating power of $CO_2$ and thus improve the extraction of polar compounds in oil seeds.[51] In fact, the co-solvent can increase the mass transfer by cleaving bonds between plant matrices and solute.[52] Moreover, the extractivity of polar compounds may be improved by a better penetration of Sc-$CO_2$ into the matrix, due to swelling of the sample matrix with a co-solvent.[45]

These results suggest that green procedures especially MeTHF had a better selectivity and gave oil with higher content of total phenolics than hexane or chloroform/methanol.

In this study, the radical scavenging activity of carvi oils was determined using DPPH assay. As shown in Table 3, carvi oils exhibited antiradical activity with $IC_{50}$ values varying from 102 to 520 μg/mL where Sc-$CO_2$ extracted oil exhibited the highest activity. Interestingly, oils extracted using MeTHF ($IC_{50}$ = 117 μg/mL) and Sc-$CO_2$ ($IC_{50}$ = 102 μg/mL) showed stronger radical scavenging activity than conventional extracted ones ($IC_{50}$ values of 248 and 520 μg/mL respectively for hexane and Folch extracted oils). Moreover, a negative correlation was found between the antiradical activity and the total polyphenols content of the oils which suggested that the antioxidant activity might be related to the presence of some individual active phenolic compounds. In fact, it was reported that the antioxidant activity not only depends on the concentration, but also on the structure and the interaction between the antioxidants.[53]

The results indicated that green procedures could be a good alternative to hexane and chloroform/methanol to recover carvi oils enriched in phenolic compounds with enhanced antioxidant activity.

## 3.5 | Anti-inflammatory activity

Firstly, carvi oils have been tested for their cytotoxic activity on macrophages, assessed by resazurin test. Our data did not show any significant cytotoxic effect for all tested concentrations (0–100 μg/mL, data not shown). Based on these data, subsequent assays were performed to elucidate the anti-inflammatory effect of these oils using LPS-stimulated RAW 264.7 macrophages.

Through their secretions and receptors, macrophages participate in complex interactions involving cellular and humoral components of the inflammatory and immunological networks. NO is among the important products of macrophage and is produced from L-arginine by inducible nitric oxide synthase (iNOS). It has been shown to play a central role in inflammatory and immune reaction activities. Stimulation of macrophages by various stimuli, such as LPS, disturb the balance of the intracellular state and leads to an excess of inflammatory mediator production such as reactive oxygen species (ROS) and NO in addition to some enzymes like iNOS.[54] NO mediates diverse physiological functions; however, excess of NO can contribute to tissue injury in inflammatory diseases. Indeed, it can react with superoxide radicals to form the harmful peroxynitrite.[55] Therefore, inhibition of NO production may be an interesting strategy to treat various inflammatory disorders.

The results presented in Figure 2 are expressed as quantity of NO production in LPS-stimulated RAW 264.7 macrophages. Our results showed that LPS treatment triggered significant nitrite accumulation in control cells. Interestingly, NO production was significantly inhibited by treatment with the four carvi oils. Indeed, hexane and chloroform/methanol oils decreased NO release in a concentration-depended manner as it is depicted in Figure 2. When tested at a low concentration (10 μg/mL), the two oils decreased NO production by macrophages by 19 and 27%, respectively, compared to control.

At 50 μg/mL, these oil exhibited strong anti-inflammatory power inhibiting NO release in LPS-induced RAW 264.7 macrophages by 81 and 87%, while at higher concentration (100 μg/mL) NO production was totally inhibited. For the two oils $IC_{50}$ were low of 28 and 24 μg/mL, respectively. In comparison, the positive control L-NAME (250 μM) used as positive control, inhibited NO release by 71%. In this work, we elucidate for the first time the anti-inflammatory effect of carvi oil.

Compared to conventional extracted oils, the green extracted ones displayed comparable strong anti-inflammatory activity. They inhibited NO release in a concentration-depended manner and displayed very low $IC_{50}$ of 27 and 28 μg/mL, respectively, in MeTHF and Sc-$CO_2$ oils.

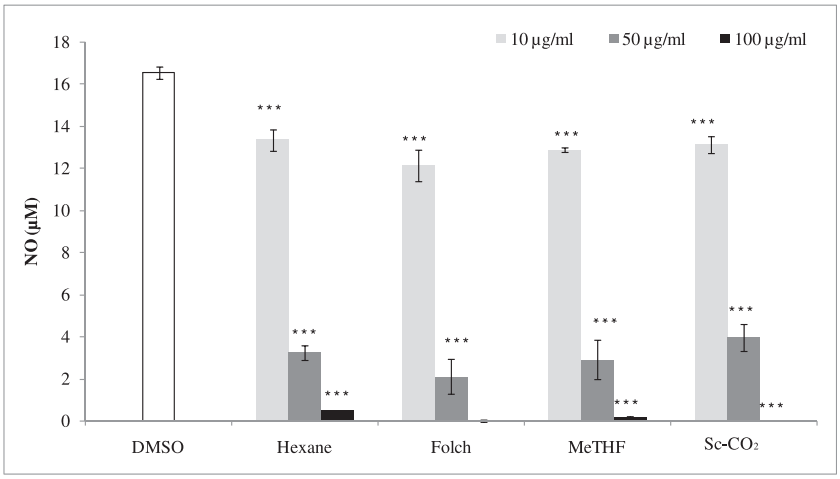


**FIGURE 2** Effect of oil of *Carum carvi* seeds obtained with conventional (Soxhlet and Folch) and green (MeTHF and Sc-$CO_2$) extractions on LPS-induced NO production in RAW 264.7 macrophages. Data are represented as mean ± SEM ($n$ = 3). ***$P$ < 0.001. NO, nitric oxide; DMSO, dimethyl sulphoxide; LPS, lipopolysaccharide; MeTHF, 2-methyltetrahydrofuran; Sc-$CO_2$, supercritical carbon dioxide; SEM, standard error of the mean

As for conventional extracted oils, treatments with elevated concentration of carvi green extracted oils (100 µg/mL) induced total NO production inhibition.

The highest anti-inflammatory power of carvi oils in our study could be due to the effect of some active compounds mainly phenolics and sterols. It has been reported that total phenolic fraction or isolated compounds from olive oil incubated with inflammatory cells down-regulated anti-inflammatory enzyme activities which induce a reduction of NO production.[56,57] Besides, sterols may have a protective effect on some mediators involved in inflammatory damage development. In fact, β-sitosterol has been found to reduce NO release in activated macrophages which was correlated with the impairment of iNOS activity.[58,59]

In this study, the replacement of conventional hazardous systems including hexane and Folch system by green procedures using Sc-$CO_2$ extraction and the bio-based, non-toxic and biodegradable solvent MeTHF have been taken up to extract oil from carvi seeds. Obtained results indicate that green extracted oils exhibited similar fatty acid composition to conventional extracted ones and were rich in UFAs mainly petroselinic acid which was the major compound. Interestingly, green extracted oils were richer than conventional extracted oils in bioactive compounds including sterols and phenolics with enhanced antioxidant and anti-inflammatory activities. In term of yield, MeTHF was more suitable than SFE to extract oil from carvi seeds giving higher yield than hexane. In conclusion, MeTHF could be a potential green industrial alternative to the petroleum solvents to obtain seed oils with high yield and quality.

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