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# Compositional and functional difference in cumin (*Cuminum cyminum*) essential oil extracted by hydrodistillation and SCFE

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**Abstract:** Essential oils were obtained from same raw material of cumin seed by extraction with hydrodistillation and super critical fluid extraction (SCFE). For SCFE, supercritical carbon dioxide at 45°C and 100 bar was used as variable for the extraction. The composition of the extracts was determined by gas chromatography-mass spectrometry. Yield of essential oil was more in the SCFE method. Extract obtained by supercritical fluid extraction technique using $CO_2$ was heavier than the hydrodistilled volatile oil. Cumin oil obtained by hydrodistillation contained higher percentage of cuminaldehyde (52.6%), then did oil obtained by SCFE (37.3%), whereas cumin oil obtained by hydrodistillation had the lower percentage of cuminic alcohol (13.3%) as compared to 19.3% in SCFE method. However, carenal (2-caren-10-al) content was almost similar in cumin oil obtained by the SCFE and hydrodistillation method (24.5–25.8%). Hydrodistilled volatile oil showed better antioxidant activity measured by DPPH and FRAP assay and more total phenol content. The results indicated that though essential oil yield was more in the SCFE method, antioxidant property was more in conventional hydrodistillation method. SCFE extracted non polar (wax materials) compounds along with volatile oil and it was recorded that enhanced aroma of signature compounds of cumin.

## ABOUT THE AUTHORS

Supradip Saha is a senior scientist at ICAR-IARI, New Delhi, India and his research interest lies in the area of nutraceuticals.

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## PUBLIC INTEREST STATEMENT

Rapidly increasing consumer demand for safe and quality food ingredients has led to search for environment friendly food ingredients. Spices have been used in foods in different cuisines for their antimicrobial activity of their constituent essential oil. Being used in food industry in different functional foods, the extraction procedure should be green in nature. Supercritical fluid extraction technology is a green and safe technology for extraction of essential oil. The present investigation deals with the quality of essential oil extracted by supercritical carbon dioxide. This technology could be a better approach to deal with the problem related to residual solvents and it could be effectively used in food industry. This "green" extraction technique will be useful for food industry.

**Subjects: Clean Tech; Food Additives & Ingredients; Food Science & Technology; Nutrition; Sensory Science**

**Keywords: supercritical fluid extraction; hydrodistillation; cumin; antioxidant activity**

## 1. Introduction

Spices are common food adjuncts, which have been used as flavoring, seasoning, and coloring agents and sometimes as preservatives throughout the world for thousands of years, especially in India, China, and many other southeastern Asian countries (Srinivasan, 2005). Not only spices are being used as food flavorings and seasoning agents, but they also be used as traditional medicines. Spices have been used in foods particularly in Indian cuisines for their varying degree of antimicrobial activity of the essential oil (Valero & Salieron 2003; Singh, Maurya, deLampasona, & Catalan, 2004). The antimicrobial activity of some essential oil components (Bullerman, Lieu, & Seier, 1977) against foodborne pathogens, including mycotoxin-producing fungi, has been developed and proposed for use in foods as natural antioxidants and antimicrobials (Hsieh, 2000). Hence, the antioxidant and antimicrobial properties of added materials are very important to improve the shelf life of food material and at the same time provide safety to consumers.

The oxidative deterioration of lipids is a great concern in the shelf life of foods. Lipid oxidation decreases food safety and nutritional quality by the formation of potentially toxic products and secondary oxidation products during cooking or processing (Maillard, Soum, Boivin, & Berset, 1996; Shahidi, Janitha, & Wanasundara, 1992). Potential health hazards of synthetic antioxidants in foods, including possible carcinogens, have been reported several times (Ford, Hook, & Bond, 1980; Hettiarachchy, Glenn, Gnanasambandam, & Johnson, 1996). The growing interest in the substitution of "traditional food preservatives," both antimicrobials and antioxidants, by natural preservatives fostered research on plant sources and the screening of plant materials in order to identify new components.

Cumin (*Cuminum cyminum* L.) is an aromatic plant belongs to Apiaceae family and is used to flavor foods, added to fragrances, and used in medical preparations (Iacobellis, Lo Cantore, Capasso, & Senatore, 2005). Its fruit, known as cumin seed, is yellow to brownish-gray in color and it contains oleoresin. It is cultivated mainly in the Middle East, India, and Pakistan. Ground cumin seeds are used commercially to flavor many ethnic cuisines (e.g. Indian, Latin American, and Mexican). Cumin commonly contains between 2 and 3.5% volatile oil (VO). It has been used in the treatment of mild digestive disorders as a carminative and eupeptic, as astringent in broncopulmonary disorders, and as a cough remedy, as well as an analgesic.

Supercritical fluid extraction (SFE) has been proven an excellent replacement for many of the classical approaches to the analysis of volatile and semivolatile analytes in natural products. The extraction of cumin volatile oil by supercritical fluid extraction (SFE) was studied by Eikani, Goodarznia and Mirza (1999), Heikes, Scott, and Gorzovalitis (2001). Li and Jiang (2004) reported a yield of 3.8% of essential oil in Chinese cumin by hydrodistillation method. It has been widely accepted by many investigators that compared favorably with hydrodistillation the SFE provides a rapid and quantitative method for extracting essential oils from aromatic plants (Hawthorne et al., 1993; Kerrola, 1995; Stahl, Quirin, & Gerard, 1988).

## 2. Materials and methods

### *2.1. Reagents and chemicals*

Solvents for GC-MS were chromatographic grade and were obtained from Merck®, India. Liquid $CO_2$ was procured from local markets of New Delhi.

### *2.2. Sample preparation and extraction*

#### *2.2.1. Supercritical fluid extraction*

Forty grams ground cumin was taken and quantitatively transferred to an extraction thimble tamped in the lower end. Tap the thimble on the bench top intermittently during transfer to effect a compact fill. Extract with supercritical $CO_2$ using SFE conditions is described below. After reaching the set pressure, allow the sample to extract statically for 30 min. Continue extraction with a 30-min dynamic segment at a flow of 30 g $min^{-1}$ $CO_2$. Collected the volatile oil, thus extracted, by rinsing the collection vessel with hexane in a 20-mL glass vial and analyzed the extracts directly using GC–MS.

#### *2.2.2. Supercritical fluid extractor*

Thar SFC with Process Suite software operating conditions: vessel heater and vessel internal temperature, cyclone heater (500 mL) set at 45°C. Pressure is 100 bar and dynamic flow of 25 g $min^{-1}$ $CO_2$. The equipment contains a pressurized $CO_2$ reservoir, a thermostatic bath kept at 5 ∘C, and a stainless steel jacketed column with 500-mL capacity and the extraction temperature was also controlled. The extraction unit also contains valves, flow regulators, and manometers for flow control.

### *2.3. Hydrodistillation*

The essential oil of the cumin seed powder was obtained by the Clavenger apparatus, using the hydrodistillation method. The dried powdered seeds of cumin (160 g) were placed in a distillation apparatus with 1 L of distilled water and hydrodistilled for three hours. The oil was then removed and passed through anhydrous sodium sulfate before storing at 4°C until analyzed.

### *2.4. Fatty acid profile*

FAMEs were obtained according to transesterification method. This method can be explained briefly as follows: the fatty acids were methylated after dissolving the sample in methanol followed by the addition of few drops of conc. $H_2SO_4$. The corresponding FAMEs were extracted with hexane by adding salt solution for complete recovery. For determination of individual components in the real sample, the prepared sample was directly injected in the GC column.

### *2.5. Gas chromatograph-mass spectrometry*

GC-MS analysis was carried out using 7890A GC (Agilent Technologies) with equipped with a HP-5MS column (30 m × 0.25 mm 0.25 µm, Agilent Co., USA) which was directly connected to a triple axis HED-EM 5975C mass spectrometer (Agilent Co., USA). The injection volume was 1 µl with flow mode in split control. The carrier gas flow was set at 1 ml $min^{-1}$ helium. Helium (High purity, New Delhi, India) was used as carrier gas at a head pressure of 10 psi. GC-MS condition for essential oil is described as follows. The oven temperature was initially held at 40°C for 1 min, hereafter the temperature was raised with a gradient of 3°C $min^{-1}$ until the temperature reached to 60°C and held for 10 min. Again the temperature was raised with a gradient of 2°C $min^{-1}$ up to 220°C and held for 1 min. Finally, temperature raised up to 280°C with increment of 5°C $min^{-1}$. Total run-time was 111 min. Other settings were as follows: 250°C interface temperature, 200°C ion source temperature, and electron impact ionization (EI) at 70 eV.

GC-MS condition for fatty acids present in waxy material of essential oil extracted by the SCFE method is described as follows. The oven temperature was initially held at 90°C for 1 min, hereafter the temperature was raised with a gradient of 5°C $min^{-1}$ until the temperature reached to 290°C and held for 5 min. Total run-time was 46 min. Mass spectra were analyzed by both full scan modes. Raw MS data were processed using the program MSD productivity Chemstation to obtain a purified spectrum by removing residual background contaminants, partially eluting peaks, and column bleed from the spectrum. Structures were confirmed using the library of the instrument. The MS acquisition parameters were: ion source 180°C, electron ionization 70 eV, full scan mode (50–550 mass units), transfer line temperature 280°C, solvent delay 3 min, and E.M voltage 889. The ionization energy was 70 eV with a scan time of 1 s and mass range of 20–500 AMU. Compounds were identified by

matching their mass spectra. NIST (National Institute of Standards and Technologies) Mass Spectra Library was used as a reference for identifying the essential components.

### *2.6. Assay*

#### *2.6.1. DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging method*

The antioxidant activity of the cumin essential compounds was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (Brand-Williams, Cuvelier, & Berset, 1995). A methanolic stock solution (50 ml) of the antioxidant (different concentrations of stock solutions) was placed in a cuvette, and 2-ml methanolic solution of DPPH was added. Absorbance measurements were taken immediately. The decrease in absorbance at 517 nm was determined by Varian Cary 50 spectrophotometer after 30 min for all samples. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidant, i.e. the control, was also measured. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula:

$$\%\text{inhibition} = ((A_C - A_t)/A_c) \times 100$$

where $A_c$ is the absorbance of the control at t = 0 min and $A_t$ is the absorbance of the antioxidant at t = 1 h.

### *2.7. FRAP assay*

FRAP was performed according to the procedure described by Benzie and Strain (1996). The FRAP reagent included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in the ratio 10:1:1 (v:v:v). Three milliliters of the FRAP reagent was mixed with 100 µl of sample extract in a test tube and vortexed in the incubator at 37° C for 30 min in a water bath. Reduction of the ferric-tripyridyltriazine to the ferrous complex formed an intense blue color which was measured at a UV–vis spectrophotometer (Varian Cary 50) at 593 nm at the end of 4 min. Results were expressed in terms of µmol Trolox $g^{-1}$.

### *2.8. Determination of total phenolics content*

For extraction of total phenolics, the homogenized samples were extracted twice with 30 ml of ethanol (80%), by stirring and sonicating for 30 min in dark. The homogenate was then centrifuged for 15 min at 10,000× g at 4°C (Eppendorf, Westbury, and U.S.A). The supernatant was then vacuum concentrated at 40°C in a rotary evaporator and stored at –20°C. The concentrated sample was used as sample extract for estimation of total phenolics and hydrophilic antioxidant activity. TPH was estimated spectrophotometrically using the Folin–Ciocalteu reagent. To the 100 µl of the sample extract (80% ethanol), 2.9 ml of deionized water, 0.5 ml of the Folin–Ciocalteu reagent, and 2.0 ml of 20% $Na_2CO_3$ solution were added. The mixture was allowed to stand for 90 min and absorption was measured at 760 nm against a reagent blank in UV–vis spectrophotometer (VARIAN Cary 50). Results were expressed as gallic acid equivalent (mg GAE 100 $g^{-1}$).

## 3. Results

### *3.1. Comprehensive comparison of the methods*

For industrial use of technology, color, texture, and extraction yield are the prime quality factors of essential oil. Besides this, extraction time and energy requirement are also important factor. Therefore, comprehensive comparisons of the clove oils obtained by hydrodistillation and SCFE method are listed in Table 1.

In Table 2, the content of cuminaldehyde, cuminic alcohol, and other terpenoid components was determined by GC-MS. The content of the main biological ingredients of essential oil of cumin by hydrodistillation is similar as the SCFE method, although its yield of the volatile oil is higher in the SCFE method. Extraction yield of SCFE was about two times as high as that obtained by

**Table 1. Characteristic of the cumin oil obtained by two different methods**

| Extraction method | Extraction period (h) | Colour, texture | Organic solvent used | Yield (%) | Spcific gravity | Refractive index | Fatty acid presents |
|---|---|---|---|---|---|---|---|
| SCFE | 0.5 | Brownish viscous oil | No | 1.71 | 0.92 | 1.48 | Palmitic acid, Oleic acid, Linoleic acid |
| Hydrodistillation | 3 | Pale yellow | Yes | 0.72 | 0.90 | 1.52 | Nil |

**Table 2. Chemical composition of the cumin oil extracted by two different methods**

| Peak No. | Compound | $R_t$ (min) | Relative peak area (%) | |
|---|---|---|---|---|
| | | | Hydrodistillation | SCFE |
| 1 | β-pinene | 12.388 | 0.24 | 0.63 |
| 2 | *p*-cymene | 16.466 | 0.97 | 1.76 |
| 3 | γ-terpinene | 20.169 | 1.85 | 4.43 |
| 4 | Cuminic aldehyde | 36.640 | 52.56 | 37.31 |
| 5 | Phellandral | 38.758 | 0.16 | — |
| 6 | Carenal (2-caren-10-al) | 40.025 | 24.53 | 25.78 |
| 7 | Cuminic alcohol | 40.489 | 13.26 | 19.31 |
| 8 | Carvacrol | 41.138 | 0.12 | — |
| 9 | γ-cadinene | 45.674 | 0.17 | 0.17 |
| 10 | β-farnesene | 50.993 | 0.23 | 0.24 |
| 11 | α-cubebene | 51.342 | 0.12 | 0.14 |
| 12 | γ-curcumene | 51.641 | 0.21 | — |
| 13 | α-logipinene | 51.622 | — | 0.19 |
| 14 | Tricosane | 93.045 | — | 0.18 |
| 15 | Eicosane | 109.599 | 0.20 | 0.22 |
| 16 | Docosane | 110.477 | 0.21 | — |

hydrodistillation. Furthermore, the extract by the SCFE method is brown ointment, which means more undesired impurities and waxy residue may existed. The hypothesis was confirmed by the presence of fatty acids in the extract (Table 1). Presence of palmitic, oleic acid, and linoleic acid was recorded in the SCFE extracted oil. Although, SCFE offers the important advantages over hydrodistillation method as the technique is milder without use of organic solvent. The highest content of cuminaldehyde in the extracted oil was obtained by hydrodistillation method. Pale yellow oil was obtained by hydrodistillation, whereas brownish viscous oil was yielded in SCFE. Additionally, using supercritical $CO_2$ instead of some harmful organic solvents would result in "greener" processes.

### *3.2. Comprehensive comparison of the composition*

Table 2 lists the composition of cumin oil obtained by hydrodistillation and SCFE methods according to the results of GC–MS. It can be seen that, 16 major terpenoids in the cumin oil have been identified, in which cuminaldehyde, carenal (2-caren-10-al), and cuminic alcohol are the main components of cumin oil. Cumin oil obtained by hydrodistillation contained higher percentage of cuminaldehyde (52.6%), then did oil obtained by SCFE (37.3%). It was reported that cuminic aldehyde showed antioxidative and antimicrobial activity with broad spectrum [18]. If the aim was to obtain high content of cuminic aldehyde in the clove oil, it may be necessary to choose hydrodistillation. Cumin oil obtained by hydrodistillation had the lower percentage of cuminic alcohol (13.3%)

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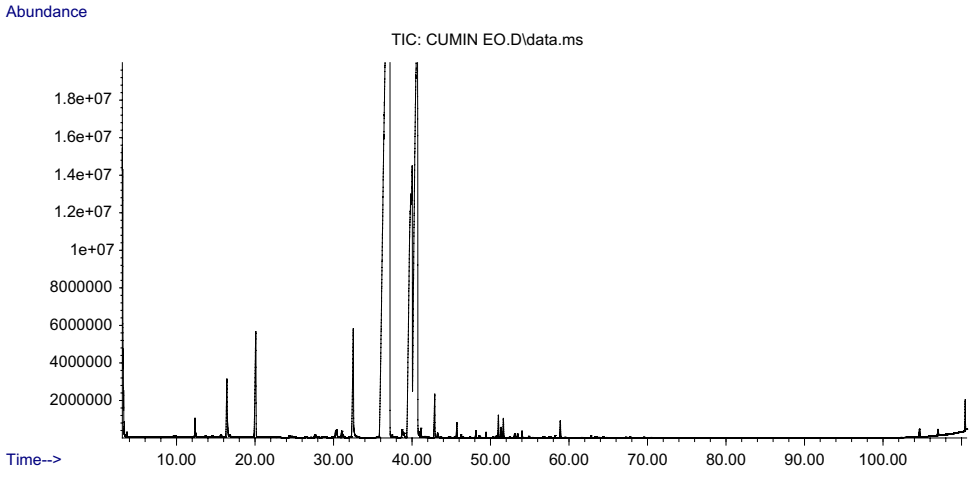
**Figure 1. TIC of cumin essential oil.**




as compared to 19.3% in the SCFE method. However, carenal (2-caren-10-al) content was almost similar in cumin oil obtained by the SCFE and hydrodistillation method (24.5–25.8%).

Besides cuminaldehyde, carenal (2-caren-10-al), and cuminic alcohol in cumin oil, relative content of γ-terpene in the cumin oil extracted by hydrodistillation and SCFE method was as high as 1.85 and 4.43%, respectively (Figure 1). Besides essential oil constituents, SCFE contains wax, resin, and other fatty materials. After analysis prior to methylation of fatty acids, palmitic, oleic, and linoleic acids were found, which was absent in hydrodistilled essential oil.

Among minor constituents, phellandral, carvacrol, and γ-curcumene were absent in essential oil extracted by the SCFE method, whereas, α-logipinene was only found in the SCF extracted oil.

### *3.3. Comprehensive comparison of the antioxidant activity*

Determination of the biological activity of plants and their extracts is one of the important areas of natural product research. Spices provide foods with flavor and food-preserving power, including antiseptic and antioxidant activity. Natural antioxidants of plant origin are becoming more and more important, not only in food, but also in preventive medicine (Risch & Chi-Tang, 1987; Larson, 1997). As many essential oils are incorporated in a large number of cuisines, it is important as well to evaluate their *in vitro* antioxidant properties. In this work, the antioxidative activity of cumin essential oil was tested using its inhibitory (protective) effect toward oxidation of reference compounds. Antioxidant activity was shown in Table 3.

Cumin oil obtained by hydrodistillation provided better antioxidant activity than the SCFE extracted volatile oil. Both the assay resulted similar results (Table 3). Essential oil extracted SCFE was 28 and 14% lower in antioxidant activity, which may be attributed to lower percentage of oil and presence of wax residues. Presence of fatty materials was confirmed by the presence of fatty acids in the SCFE extracted oil, which is absent in hydrodistilled oil. Similarly, total phenolic content was more in hydrodistillation method. Total phenolics content was 10.2 µg GAE $mg^{-1}$ in hydrodistillation method as compared to 9.3 µg GAE $mg^{-1}$.

**Table 3. Antioxidant activity of cumin essential oil by two different methods**

| Extraction method | Antioxidant activity | | Total phenolics (µg GAE $mg^{-1}$) |
|---|---|---|---|
| | FRAP (µmol trolox $g^{-1}$) | DPPH (µmol trolox $g^{-1}$) | |
| Hydrodistillation | 0.064 | 0.28 | 10.2 |
| SCFE | 0.046 | 0.24 | 9.3 |

## 4. Discussion

Eikani et al., (1999) compared the two extraction techniques (conventional steam distillation vs. SCFE) of essential oil of cumin and it was concluded that the method could extract valuable components by the supercritical procedure, which would otherwise be thermally degraded by conventional steam distillation. On the other hand, Li, Tian, Pang, Shi, and Feng (2009) concluded that organic solvent with low boiling point and steam distillation was considered better than SCFE and other methods for obtaining high-quality *C. cyminum* essential oil. However, the study is in contrast to the experiment by Guan, Li, Yan, Tang, and Quan(2007). SFE is considered as the optimum process for obtaining high-quality clove oil.

Hawthorne et al.,(1993) concluded that SFE recovered $C_{27}$, $C_{29}$, $C_{31}$, and $C_{33}$ *n*-alkanes from three essential oil sources that were not otherwise extracted by hydrodistillation. But our result is not in agreement with the study. Tricosane and eicosane were present in the SCF extracted oil, whereas, eicosane and docosane were recorded in hydrodistilled oil. Reverchon and Senatore (1992) compared hydrodistillation and SCFE and it was found that although roughly the same compounds were extracted, the two oils of rosemary possessed a widely different percentage composition. Qualitative aroma testing showed that the oil obtained by SFE using $CO_2$ showed a fragrance that better resembled that of the rosemary leaves used for the isolation of the oil. Our observations are also similar to the study.

In the present experiment, cuminic aldehyde was major constituent in the essential oil. Concentration was 52.6 and 37.3% in the hydrodistillation and SCFE method, respectively. Other major constituents were carenal (2-caren-10-al) (24.5–25.8%) and cuminic alcohol (13.3–19.3%). Cuminic aldehyde was less in SCFE, but the other major constituents were more in SCFE. Li and Jiang (2004) reported that seeds of cumin from china constitute carenal (2-caren-10-al) (36.31%), cuminic alcohol (16.92%), $\gamma$-terpinene (11.14%), safranal (10.87%), *p*-cymene (9.85%), and $\beta$-pinene (7.75%) as major components. Similar observations were reported by Uhl (2000). The study reported cuminic aldehyde (33%), β-pinene (13%), terpinene (29.5%), *p*-cymene (8.5%), *p*-menthon-1,3-dien 7-al (5.6%), cuminyl alcohol (2.8%), and β-farnesene (1.1%) (Uhl, 2000). Similar observation was reported by Viuda-Martos, Ruiz-Navajas, Fernandez-Lopez, and Perez-Alvarez (2007) except the presence of cuminic alcohol whereas Baser, Kürkçüoglu, and Özek (1992) and Beis, Azcan, Ozek, Kara, and Baser (2000) reported p-mentha-l,3-dien-7-al and p-mentha-l,4-dien-7-al were also contributes to the major constituent of cumin essential oil apart from cuminaldehyde, α-terpinene, p-cymene, and β-pinene. Li and Jiang (2004) reported that essential oil of cumin includes cumin aldehyde, cuminic alcohol, γ-terpinene, safranal, paracymene, and β pinene.

Besides essential oil, wax, lipid, and resin were also extracted by SCFE. Presence of fat was confirmed by the presence of palmitic, oleic, and linoleic acids in the SCFE extracted oil. Major fatty acid was oleic acid and palmitic acid was least in concentration. It can also be seen that the oil obtained by SCFE contains small amount of co-extracted cuticular waxes, which contributed to the viscosity of the extracted oil. This result is similar to other author's work (Guan et al., 2007; Mostafa, Yadollah, Fatemeh, & Naader, 2004; Myint, Wan Daud, Mohamad, & Kadhum, 1996).

These properties are also very much needed by the food industry in order to find possible alternatives to synthetic preservatives (namely BHT, phenolics). In this context, *Cuminum cyminum* essential oils, gave interesting results, being one of the promising performing extracts in terms the ability to neutralize free radicals and prevent unsaturated fatty acid oxidation. The results presented here may also contribute to knowledge of the antioxidative potentials of these species reported elsewhere.

Antioxidant activity was reported earlier by many researchers (Allahghadri et al., 2010; Gachkar et al., 2007). Allahghadri et al., 2010 reported that total phenol content of the essential oil was estimated to be 33.43 µg GAE $mg^{-1}$ of the oil. Hexane extract of cumin showed total phenolic content of 10.6 mg $g^{-1}$ dry extract (El-Ghorab, Nauman, Anjum, Hussain, & Nadeem, 2010). In contrast to our

study, Tipsrisukond, Fernando, & Clarke, 1998 reported that essential oil prepared by a conventional method was less effective as antioxidants than ground black pepper, oleoresin extracted by SCF-$CO_2$.

## 5. Conclusions

Significant difference was observed between essential oil extracted by hydrodistillation and supercritical fluid extraction in yield and physical characteristics. In chemical profiling, variation in content of three major terpenoides cuminal, carenal (2-caren-10-al) and cuminic alcohol was also recorded, which is reflected in antioxidant activity. Hydrodistilled essential oil was found more active than SCFE method. As yield was double the amount extracted by the hydrodistillation method, activity may be compensated as the extraction process is green and ecologically sound.

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**Competing interests**
The authors declare no competing of interests.

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