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Siti Machmudah[a]; Yuko Shiramizu[a]; Motonobu Goto[a]; Mitsuru Sasaki[a]; Tsutomu Hirose[a]
[a] Department of Applied Chemistry and Biochemistry, Kumamoto University, Kumamoto, Japan

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# Extraction of *Nigella sativa L.* using Supercritical $CO_2$: A Study of Antioxidant Activity of the Extract

**Siti Machmudah, Yuko Shiramizu, Motonobu Goto, Mitsuru Sasaki, and Tsutomu Hirose**
Department of Applied Chemistry and Biochemistry, Kumamoto University, Kumamoto, Japan



**Abstract:** Oil from *Nigella sativa* seed has been extracted by supercritical $CO_2$ as a solvent at various pressures. In this study, two conditions of separation were used; they were low and high pressure separation. The antioxidant activity of the oil extracted was measured using an ultraviolet-visible spectrum (UV-Vis) spectrophotometer. There was no effect on the change of extraction pressure in apparatus with low pressure. The essential oil content in the extract from apparatus with high pressure was approximately two times higher than it was with low pressure. The antioxidant activity of *Nigella sativa* oil showed positive result. The antioxidant activity was obtained by the quantity of thymoquinone and carvacrol.

**Keywords:** Supercritical $CO_2$ extraction, *Nigella sativa L.*, thymoquinone, antioxidant activity

## INTRODUCTION

The seeds of *Nigella sativa*, commonly known as black cumin, and kalunji have been extensively investigated in recent years. This seed has a rich historical and religious background. In the Middle East, Northern Africa, and India it has been used traditionally for centuries for the treatment of asthma, cough,

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Address correspondence to Motonobu Goto, Department of Applied Chemistry and Biochemistry, Kumamoto University, Kurokami 2-39-1, Kumamoto 860-8555, Japan. Fax: +81-96-342-3679; E-mail: mgoto@kumamoto-u.ac.jp

bronchitis, headache, rheumatism, fever, influenza, and eczema, and as a diuretic, lactagogue, and a vermifuge. Furthermore, black cumin seeds are of importance as a carminative and spice, often they are used as a condiment in bread and other dishes (1).

The black seeds, known in Arabia as "Habbah Sauda," "Habbet el Baraka," "Kamun-aswad," and "Shunez," contain 36%–38% fixed oil, proteins, alkaloids, and saponins and 0.4%–2.5% essential oil (2). The fixed oil is composed mainly of unsaturated fatty acids, including unusual C20 : 2 arachidic and eicosadienoic acids. The essential oil was analyzed by Burits and Bucar (1) using gas chromatography–mass spectrometry (GC–MS). Many components were characterized, but the major ones were thymoquinone (27.8%–57.0%), r-cymene (7.1%–15.5%), carvacrol (5.8%–11.6%), t-anethole (0.25%–2.3%), 4-terpineol (2.0%–6.6%), and longifoline (1.0%–8.0%).

Recently, many medical properties have been attributed to the black cumin seeds and/or its oil, such as antioxidant (1), anticarcinogenic (antitumor) (3), antibacterial (4), anti-inflammatory, analgesic, and antipyretic (5). Further studies indicated the presence of antihepato, nephrotoxic, and antiparasitic activities (6, 7). Also black cumin seeds can affect the cardiovascular system and the blood (8).

Until now only a few investigators have isolated nigella seed oil. All of them used conventional methods, such as hydro-distillation and solvent extraction. Burits and Bucar (1) isolated nigella seed oil using hydro-distillation and soxhlet extraction. Through the hydro-distillation process, the content of thymoquinone was only 3% instead of 48% obtained by soxhlet extraction. Atta (9) has extracted nigella seed oil using petroleum ether at 40–60°C. He revealed that nigella seed is a good source for oil and protein. He has also investigated the oil properties. Ramadan and Morsel (10) extracted nigella seed oil with two different solvents, n-hexane and a mixture of chloroform/methanol.

Supercritical fluid extraction (SFE) is an attractive alternative to conventional methods due to its use of environmentally compatible fluids, reduced solvent consumption, oxygen-free extraction environment, the ease of separation of solute from supercritical fluid (SCF) solvent by simple expansion and shorter extraction time. Supercritical $CO_2$ extraction has been considered as a possible applied field of SFE, because $CO_2$ is nontoxic and nonflammable, the lack of a chemical residue problem and low critical temperature (31.2°C) is important.

The purpose of this study was to extract nigella seed oil using supercritical $CO_2$ at various pressures and then examine the anti-oxidant activity of extract.

## EXPERIMENTAL

### Materials

Nigella seeds (*Nigella sativa L.*) were purchased from local markets in Egypt. The seeds were ground before extraction in a coffee grinder to enhance

extraction efficiency and then were stored at room temperature. Chemicals for antioxidant activity analysis were purchased from Wako Pure Chemical Ind., Japan.

## Methods

The schematic flow diagrams of the extraction apparatus are shown in Figs. 1 and 2. In Fig. 1, the apparatus includes a separator (273 K, 0.1 MPa) (SFE 1), while in Fig. 2, the apparatus includes a high pressure separator (273 K, 2.5 MPa) (SFE 2).

About 5 g of nigella seeds were placed in the extractor. Nigella seed oil was extracted with supercritical carbon dioxide. Liquid carbon dioxide from a cylinder with a siphon attachment was passed through a chiller (SC-600, Iuchi, Japan) and was then compressed to the operating pressure by a syringe pump (100DX syringe pump by ISCO, USA). Compressed $CO_2$ flowed into the 10 mL capacity extractor vessel in a heating chamber (ST-110, Espec Co., Japan) that controlled the extraction temperature. The exit fluid from the extractor was expanded in the separator by a back-pressure regulator, which was heated by a ribbon heater to prevent it from freezing. The solute extracted was collected in the separator, and the $CO_2$ flow rate was measured by a gas flow meter. The pressure in the extractor and separator (SFE 2) was controlled by the back-pressure regulator located at the exit of the column.

In this study, extractions were carried out at optimum temperature of 313 K, pressures of 20–50 MPa, $CO_2$ flow rate of 2 mL/min, and extraction time of 170 min.



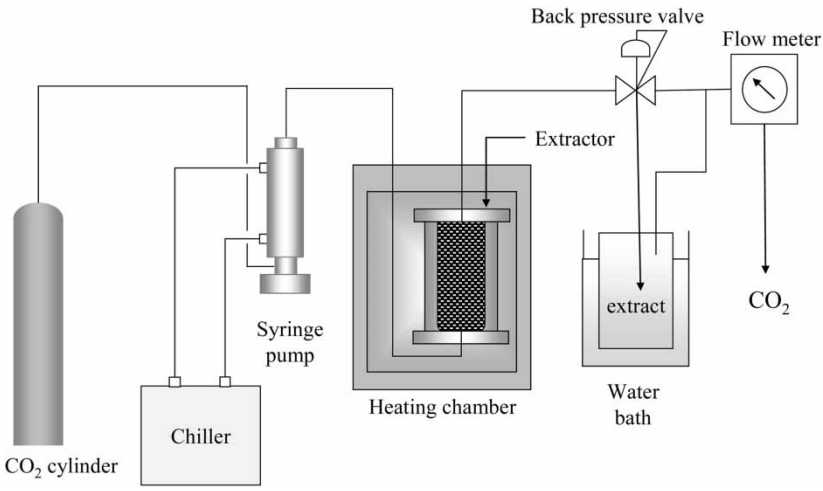


***Figure 1.*** Schematic diagram of one stage SFE apparatus (SFE 1).

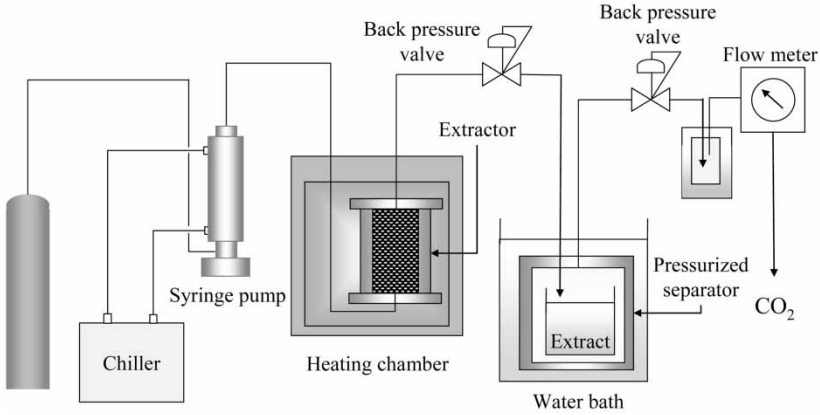


***Figure 2.*** Schematic diagram of SFE apparatus using high pressure separator (SFE 2).



## Analysis

Extracts were analyzed using a GC–MS (Hewlett Packard 6890) coupled with a mass selective detector (HP 5973). The oven temperature program was used between 343–503 K. The carrier gas was helium. In this study, Gas Chromatography Flame Ionization Detector (GC FID) (GC-14B, Shimadzu) with a DB-Wax capillary column (J&W Scientific, 15 m × 0.250 mm × 0.25 μm) also was used to analyze the extract. The oven temperature was similar with to GC–MS condition.

## Antioxidant Activity

The antioxidant activity was measured using the methodology of Hammerschmidt and Pratt (11) modified by Zancan, et al. (12) The reaction substrate was prepared using 10 mg of $\beta$-carotene, 10 mL of chloroform, 60 mg of linolenic acid, and 200 mg of Tween 80. This solution was concentrated in a rotary evaporator (Buchi, Switzerland) at 50°C and afterward diluted with 50 mL of distilled water. The reaction was conducted using the following procedure: to 1 mL of substrate 2 mL of distilled water and 0.05 mL of extract diluted in ethanol (0.02 g of extract/1 ml of ethanol) were added. The mixture was set into a water batch (Water Batch BM100, Yamato, Japan) at 40°C and the reaction product was monitored using a UV-Vis Spectrophotometer (Jasco V-550, Japan) for 0, 1, 2, and 3 h at 470 nm. Furthermore the antioxidant activity of extract was compared to $\alpha$-tocopherol as calibration.

## RESULTS AND DISCUSSION

The GC–MS analysis of the commercial essential oil of *Nigella sativa* is shown in Fig. 3. The main fraction of the essential oil consisted of a mixture of monoterpenes. The main compounds were p-cymene, thymoquinone, γ-terpinene, and carvacrol. The quality of antioxidant is obtained by thymoquinone and carvacrol (1). Figure 4 shows the GC–MS analysis of essential oil extracted. Tetralin was added to the essential oil as an internal standard for quantitative analysis.

Figure 5 shows the effect of the separator mode of equipment on the extraction yield. In this study, yield is defined as weight of extract divided by weight of sample. The extraction yield in SFE 2 is higher than is the other one for 30 MPa, and almost the same as the extraction yield in SFE 1 for 40 and 50 MPa. The extraction yield increased with increasing pressure at 20–30 MPa, and then decreased with increasing pressure. It can be explained that at 20–30 MPa the extraction process was dependent on the solvent density, while at 40 and 50 MPa the solute vapor pressure was more effective than solvent density.

The contents of compounds in the extract for SFE 1 and SFE 2 are shown in Table 1. For SFE 1 the content of extract was almost constant and independent of pressure. The content of commercial essential oil is higher than that of the oil extracted. While in SFE 2, the content of extract depended on the extraction pressure. The content of essential oil in extract was almost similar to commercial essential oil at 20 MPa, and the highest yield was 2.5%. The highest selectivity of thymoquinone was also reached at this condition.



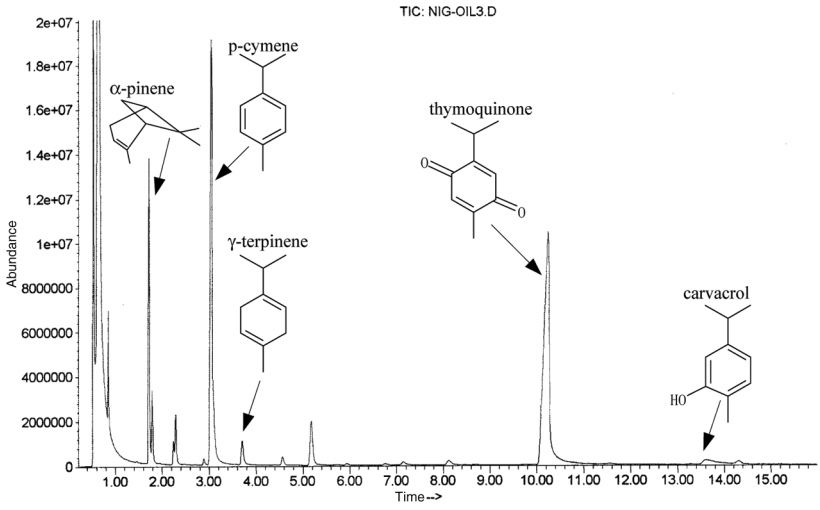


***Figure 3.*** Profile of GC-MS analysis for commercial essential oil of nigella sativa.

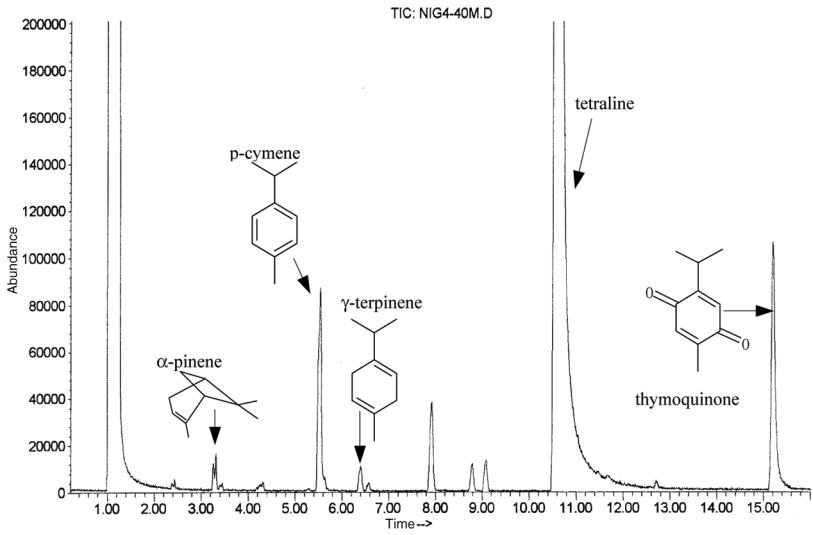


***Figure 4.*** Profile of GC-MS analysis for essential oil extracted of nigella sativa.

The measurement of antioxidant activity of *Nigella sativa* oil for SFE 2 is shown in Fig. 6. The antioxidant activity was expressed as a percentage of absorbance, and $\alpha$-tocopherol was used as a comparison. The antioxidant activity of *Nigella sativa* oil showed a positive result, although the highest activity is only 0.14 of $\alpha$-tocopherol's activity. The antioxidant activity of

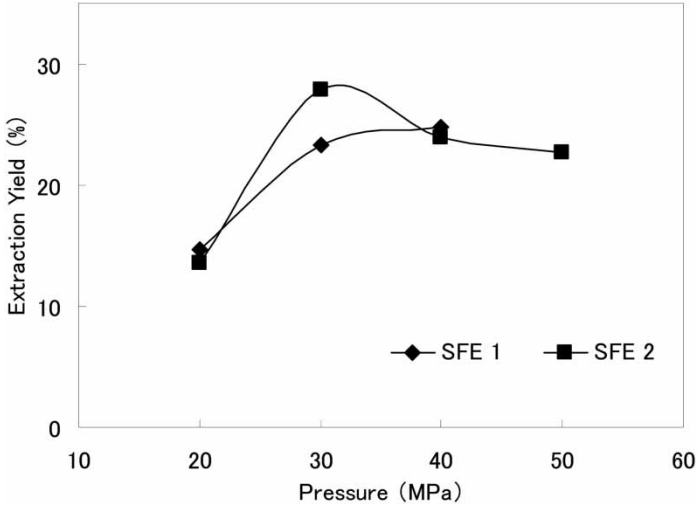


***Figure 5.*** The effect of separator mode on the extraction yield.

***Table 1.*** The content of compounds in the extract

| Compound | Commercial oil (%) | SFE 1 (%) | | | SFE 2 (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | 20 MPa | 30 MPa | 40 MPa | 20 MPa | 30 MPa | 40 MPa | 50 MPa |
| Essential oil | 2.5 | 0.65 | 0.65 | 0.79 | 2.5 | 0.67 | 0.88 | 1.14 |
| Thymoquinone | 0.95 | 0.28 | 0.30 | 0.32 | 1.42 | 0.28 | 0.40 | 0.49 |
| p-cymene | 0.39 | 0.05 | 0.09 | 0.09 | 0.19 | 0.17 | 0.15 | 0.23 |

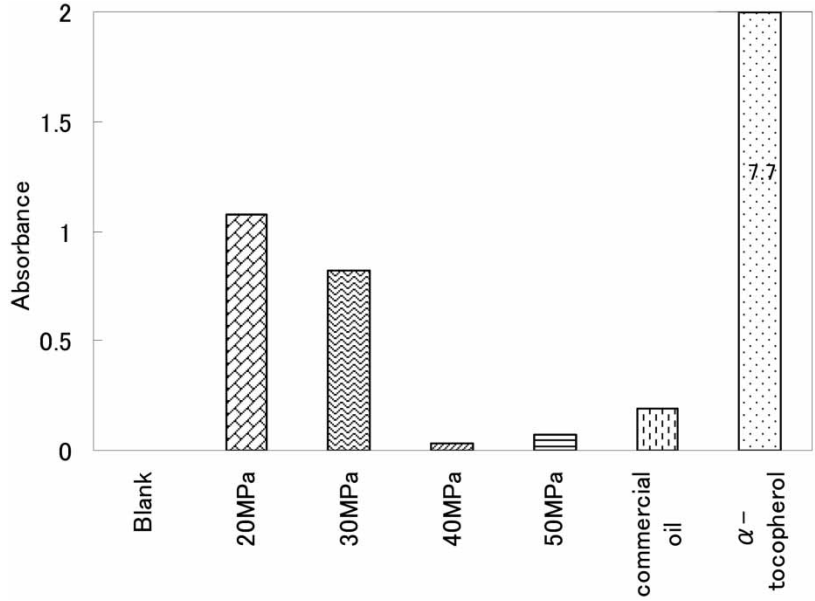


***Figure 6.*** Antioxidant activity of nigella sativa oil.

*Nigella sativa* oil at 20 and 30 MPa, respectively, are about 5 and 4 times higher than it is in commercial essential oil. It showed that the antioxidant activity not only depended on thymoquinone content, but it also depended on other components, such as carvacrol. Unfortunately, in this study carvacrol could not be traced by GC analysis because the composition of carvacrol in the extract was too small.

## CONCLUSION

Supercritical carbon dioxide extractions on nigella seed (*Nigella sativa L.*) were carried out using two separation modes of SFE apparatus. As the result, the extract was not effected by the change of extraction pressure in SFE 1, while the extract depended on extraction pressure in SFE 2. The extract of SFE 2 was higher than that of SFE 1. The antioxidant activity of Nigella sativa oil showed a positive result. The antioxidant activity was obtained by the quantity of thymoquinone and carvacrol.

## ACKNOWLEDGMENT

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