

Full Length Research Paper

Comparison of the supercritical fluid extraction with conventional extraction methods to determine the fatty acid composition of black cumin seeds

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The purpose of this study was to compare different methods of extraction to determine the fatty acid composition of black cumin seeds. 100 g of the powdered sample either was subjected into SFE equipment using standard procedures or extracted using conventional techniques (methanol or hexane solvents). Identification of fatty acid composition of extracted *Nigella sativa* oil was performed by using gas chromatography equipped with flame ionization detector (GC/FID). The constituents were identified by comparison of their retention times with those of reference samples. A total of fifteen fatty acids were identified in the fixed oils obtained from black cumin seeds by using SFE and solvent extraction techniques. The results showed that the most abundant fatty acid present in all extracts was linoleic acid. Surprisingly, despite of high potential of SFE technique in obtaining valuable components of herbs, only conventional methods were able to yield some rare fatty acid in NS such as Gamma linolenic acid. Based on condition, the researchers have to choose the method of extraction in order to obtain the best results and certain component.

Key words: *Nigella sativa*, supercritical fluid extraction, solvent extraction, gas chromatography, fatty acid composition.

INTRODUCTION

A large number of medicinal plants and their purified constituents have been shown beneficial therapeutic potentials. Seeds of *Nigella sativa*, a dicotyledonous of the Ranunculaceae family have been employed for thousands of years as a spice and food preservative. Black cumin is the dried ripe seeds of *Nigella sativa* (NS), strongly aromatic when crushed, reminiscent of anise or nutmeg, also slightly bitter tasting at first, then spicy and somewhat pungent. It contains fixed, volatile oils which contain thymoquinone and several monoterpenes

including p-cymene a-pinene (El-Tahir et al., 1993). The seeds of the *N. sativa* plant have been used to promote health and fight disease for centuries especially in the Middle East and in Southeast Asia. In South Asia, it is called *kalonji*; its Arabic name is *habat-ul-sauda* and in English it is known as black cumin. Many therapeutic effects of NS extracts have been documented including immunomodulative (Boskabady and Farhadi, 2008), anti-inflammatory (Al-Ghadmi et al., 2001), antitumor (Ait et al., 2007; Majdalawieh et al., 2010), antidiabetic (Benhaddou-Andaloussi et al., 2010) and antiulcerogenic (El-Dakhkhny et al., 2002) as well as reproductive effects (Parhizkar et al., 2011) in both clinical and experimental studies. This plant has been a focus of much research. It has several traditional uses and

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consequently has been extensively studied for its chemical constituents and biological activities. Supercritical fluid extraction of lipid has received attention as an alternative method to organic solvent extraction and has been shown to be an ideal method for extracting and fractioning oils (Vagi et al., 2002). Supercritical CO₂ is non-toxic, non-flammable and simple to use when compared to conventional organic solvents. Furthermore, SFE fractionation allows the pool of target compounds in the oil fraction. These advantages may make supercritical CO₂ extraction ideal in the food and pharmaceutical industries (Pourmortazavi and Hajimirsadeghi, 2007). To our knowledge, the comparison between different methods of extraction particularly for fatty acid profile of *N. sativa* has not yet been done, though they would provide much information about the extraction.

The availability of such data would facilitate the selection of the most suitable methods of extraction and the determination of their quality. This investigation was undertaken to obtain information about the chemical composition of *N. sativa* seeds to determine fatty acid profiles obtained by 'supercritical fluid extraction' as well as conventional extraction methods.

MATERIALS AND METHODS

Plant materials and extractions

N. sativa seeds (imported from India) were purchased from a local herb store in Serdang, Malaysia. Voucher specimens of seeds were kept at the Cancer Research Laboratory of Institute of Biosciences and the seed was identified and authenticated by Professor Dr. Nordin Hj Lajis, Head of the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia. After cleaning the seeds under running tap water for 10 min, they were rinsed twice with distilled water and air-dried in an oven at 40°C overnight until a constant weight was attained. The seeds were grounded to a powder shape using an electric grinder (National, Model MX-915, Kadoma, Osaka, Japan) for 6 min. Homogenized and grounded samples (100 g) were soaked overnight with solvents at a ratio of 1:5 (w/v ratio). Two different solvents were used: n-hexane (Pu: 99%, Merk, Darmstadt, Germany) and methanol (Dauksas et al., 2002). The mixture of sample and solvent were covered with aluminum foil and were shaken using a 'shaking incubator' (Heidolph Unimax 1010, Germany) at 5 to 7 rpm for 90 min. Then solvents were filtered using Whatman No. 1. The residues were re-soaked with fresh solvent two times to ensure the complete extraction of the oil. Solvents were completely evaporated using a rotatory evaporator (Heidolph Laborata, Germany) at 50°C and 90 rpm that yielded a blackish-brown and yellowish concentrates for methanol and hexane extract respectively which kept at -20°C prior to use. The extractive values (w/w %) of the methanol and hexane extract were 29 and 33%, respectively. Extraction of essential oil from the seed of *N. sativa* also was done using the speed supercritical fluid extraction (SFE) instrument according to the method described by Irmak (2006). The seed powder of *N. sativa* was measured to 150 g with digital scale (Shimadzu Model, Japan) balance before placing in the extraction vessel. The oil extract was obtained at 60 MPa and 40°C by means of 'supercritical fluid extraction set' (SFE-1000F Thar US Technology, USA).

SFE flow-rates were maintained at 20.00 ml/min using a variable flow restrictor. The yellowish-brown color yield was collected within

3 h and its value (w/w %) was 26% which was stored at -20°C prior to use. The collected pressure and temperature were 0.1 MPa and 25°C, respectively. The extraction was carried out with pure CO₂.

Chemicals

All solvents were of analytical grade. Methanol and hexane were obtained from Merck (Merck, Darmstadt, Germany).

Analyses of oil extract

Total lipid extract of the different extracts of *N. sativa* were carried out according to the method of Folch et al. (1957) as described by Rajion et al. (2000). Fatty acid composition was identified by gas-liquid chromatography after derivatisation to fatty methyl esters (FAMES) with 2 M KOH in methanol at room temperature according to the IUPAC standard method (IUPAC, 1992). Analyses of FAMES were carried out with a Hewlett-Packard 5890 Series II gas chromatograph (H.P. Co., USA) equipped with a hydrogen flame ionisation detector and a capillary column: HP Inovax cross-linked PEG (30 m · 0.32 mm · 0.25 µm film). The column temperature was programmed from 100 to 190°C at 5°C/min and the injector and detector temperatures were set at 220°C increment to facilitate optimal separation. Identification and quantification of FAMES was accomplished by comparison of their retention times with those of authentic reference standard available in the laboratory purchased from Sigma (USA) and analyzed under same conditions. Peak areas of triplicate injections were measured with a HP computing integrator. The results were expressed as a percentage of individual fatty acids in the lipid fraction. All measurements were done in triplicate and the average was considered as final results.

Statistical analyses

Statistical analysis was carried out using SPSS for windows program version 15 (SPSS Institute, Inc., Chicago, IL, USA). Data were subjected to analysis of variance (ANOVA) and where significant differences were observed, means were further subjected to Duncan's multiple range test. A $p < 0.05$ are considered as significant differences.

RESULTS

The fatty acid compositions (% of total fatty acid) of different extracts of NS are shown in Table 1. The most abundant fatty acid present in NS methanol, SFE and hexane extract was linoleic acid (C18:2), which gave 56.82, 52.66 and 53.14% of total fatty acid respectively. The other major fatty acids was oleic acid (C18:1), which produced the percentage of total fatty acid 24.40, 22.74 and 23.04 in methanol, SFE and hexane extracts respectively. From the results obtained, the major fatty acids of *N. sativa* oil were palmitic acid (10.51, 12.35 and 11.66 in M, SFE and H respectively), and stearic acid (2.91, 3.07 and 3.08 in M, SFE and H respectively) as saturated fatty acids. Furthermore, linoleic acid (56.82, 52.66 and 53.14% in M, SFE and H respectively), and oleic acid (24.40, 22.74 and 23.04 in M, SFE and H respectively) were the main unsaturated fatty acids. The total amounts of saturated fatty acid were 15.38, 21.31

Table 1. Yield and fatty acid percentage composition of three different extracts of *Nigella sativa*.

Fatty acid profile		Retention time	Type of extract		
			M	M	M
C10:0	Pentadecanoic	4.15	0.42±0.19	1.17±0.59 ^a	0.86±0.04 ^a
C12:0	Lauric	7.19	0±0	0.03±0.47 ^a	0.22±0.07 ^a
C14:0	Myristic	10.20	0±0	0 ±0 ^a	0.26±0.01 ^b
C16:0	Palmitic	13.83	10.51±0.03	13.02±1.23	11.62±0.21 ^{ab}
C16:1	Palmitoleic	15.22	0.09±0.1	0.15±0.13 ^a	0.13±0.11 ^a
C17:0	Heptadecanoic	16.32	1.17±0.63	2.7±2.34 ^a	3.71±0.55 ^a
C18:0	Stearic	19.59	2.91±0.07	3.40±0.62 ^a	3.08±0.02 ^a
C18:1	Oleic	21.33	24.42±0.17	23.09±0.66	23.02±0.16 ^b
C18:2	Linoleic	23.74	56.85±0.26	52.68±0.04	53.10±0.16
C18:3	γ- Linolenic	24.03	0.20±0.01	0 ±0	0.19±0.07 ^c
C20:0	Arachidic	25.57	0.26±0.02	0.47±0.15	0.33±0.01 ^b
C20:1	Eicosenoic	26.68	0.33±0.005	0.25±0.22 ^a	0.38±0.1 ^a
C20:2	Eicosadienoic	26.75	2.79±0.66	2.67±0.41 ^a	2.83±0.26 ^a
C20:3	Eicosatrienoic	27.65	0±0	0.03±0.45 ^a	0±0 ^a
C22:1	Erucic	33.01	0±0	0±0 ^a	0.23±0.08 ^b
TSFA	Total saturated fatty acid		15.29±0.7	21.06±0.45	20.05±0.3 ^b
TUFA	Total unsaturated fatty acid		84.67±0.7	78.93±0.47	79.9±0.27 ^b
PUFA	Polyunsaturated fatty acid		59.83±0.43	55.14±0.31	56.13±0.11 ^b
MUFA	Monounsaturated fatty acid		24.84±0.27	23.5±0.33 ^a	23.77±0.31 ^a
U:S ratio	TUFA:TSFA		5.53	3.74	3.98
P:S ratio	PUFA:TSFA		3.91	2.61	2.79

Values were determined by GC-FID of triplicate samples. M: methanol extract; SFE: supercritical fluid extraction; H: hexane extract; TSFA = total saturated fatty acid; TUFA = total unsaturated fatty acid; PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid; TUFA:TSFA = the ratio of total unsaturated fatty acid and total saturated fatty acid; ab: comparison of the means between row of the same fatty acid significant at $p < 0.05$. a means comparison with M extracts and b with SFE.

and 20.05 in M, SFE and H respectively, whereas total unsaturated fatty acids in the three different extracts were 84.62, 78.68 and 79.94 in M, SFE and H respectively.

DISCUSSION

Since the role of unsaturated fatty acids in improvement of reproduction performance have well known in human and animals, and considering efficacy of *N. sativa* and its various extracts in modulating estrogenic and metabolic effect in OVX rats, have analyzed different extracts for determining the abundant of fatty acid in *N. sativa*. From the results obtained, the dominating fatty acid was linoleic acid which accounted for more than 56% of the total fatty acids that revealed similar results with those reported by Nikavar et al. (2003) and Tulukcu (2011). This is nutritionally desirable. The high concentration of linoleic acid in black cumin seed oil is the cause of the high nutritional value of these oils as linoleic acid is one of the three essential fatty acids (Matthaus and Musa, 2011). Gomaa and Ramadan (2006) have mentioned that the nutritional value of linoleic acid is due to its metabolism at tissue levels which produces the hormone-like

prostaglandins. The activity of these includes lowering of blood pressure and constriction of smooth muscle; relief of nasal congestion and asthma and prevention of gastric ulcers (Bergstrom et al., 1968; Samuelsson, 1972). The second major fatty acid was oleic acid. The ratio of linoleic acid to oleic acid was more than 2.3:1 in the three samples which was in accordance with other studies (Atta, 2003; Ramadan and Moersel, 2003). These results also agreed with those of soybean oil (C18:2 = 52%, C18:1 = 25%) and in corn oil (C18:2 = 58.7%, C18:1 = 26.6%) (Al-Jassir, 1992). The contents of the saturated fatty acids are comparatively lower. However, the unsaturated fatty acids amounted to more than 84, 78 and 89% (in M, SFE and H extract respectively) of the total fatty acid content of the lipid extract, and all of the unsaturation was due to C18 acids. The results of this investigation agree with those reported by Babayan et al. (1978) and Al-Jassir (1992). Total saturated fatty acids were 15.38, 21.31 and 20.05% in M, SFE and H respectively. The findings of this study are different than those reported by Atta (2003) which reported as 29.2 for total saturated and 69.7 for total unsaturated fatty acids.

The present study also confirmed the existence of eicosadienoic acid and Gamma Linolenic acid in NS oil

which was in line with other studies previously (Al-Jassir, 1992; Nergiz et al., 1993; Ghanya, 2005). The current results were higher than those reported by Atta (2003) and Ghanya (2005), especially in unsaturated fatty acid content.

Conclusion

The results illustrated that the most abundant fatty acid in all extracts was linoleic acid and its concentration in methanol extract was higher than other extracts. Based on condition, the researchers have to choose the method of extraction in order to obtain the best results and certain component. The present study showed that solvent extraction especially methanol could extract higher concentration of fatty acids either abundant fatty acids (for example, linoleic acid, linolenic acid) or rare fatty acids (for example, gamma linolenic acid) compare to 'supercritical fluid extraction' which was expected higher ability to free fatty extraction. Therefore, developing alternative extraction methods with better selectivity and efficiency are highly desirable and more studies are needed to introduce an alternative; faster and cheaper method in the pharmaceutical and food field.

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