

Comparative analysis of essential oil composition of Iranian and Indian *Nigella sativa* L. extracted using supercritical fluid extraction and solvent extraction

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Abstract: The objective of this study was to compare the oil extraction yield and essential oil composition of Indian and Iranian *Nigella sativa* L. extracted by using Supercritical Fluid Extraction (SFE) and solvent extraction methods. In this study, a gas chromatography equipped with a mass spectrophotometer detector was employed for qualitative analysis of the essential oil composition of Indian and Iranian *N. sativa* L. The results indicated that the main fatty acid composition identified in the essential oils extracted by using SFE and solvent extraction were linoleic acid (22.4%–61.85%) and oleic acid (1.64%–18.97%). Thymoquinone (0.72%–21.03%) was found to be the major volatile compound in the extracted *N. sativa* oil. It was observed that the oil extraction efficiency obtained from SFE was significantly ($P < 0.05$) higher than that achieved by the solvent extraction technique. The present study showed that SFE can be used as a more efficient technique for extraction of *N. Sativa* L. essential oil, which is composed of higher linoleic acid and thymoquinone contents compared to the essential oil obtained by the solvent extraction technique.

Keywords: *Nigella sativa* L., essential oil extraction, supercritical fluid extraction, solvent extraction, fatty acid composition, thymoquinone, linoleic acid

Introduction

Nigella sativa, an annual flowering plant of Ranunculaceae family, has been used in traditional medicine throughout Middle Eastern countries, such as Asia, Africa, and India, for various ailments. It is commonly used to treat asthma,^{1,2} but a recent study of *N. sativa* demonstrated the multiple mechanisms of actions for a range of therapeutic uses. These include its use in the remedy to treat hypercholesterolemia in menopausal women, with more benefits and less side effects than from other single pharmacological agents.³

The results of another study showed that methanolic extract of *N. sativa* seed inhibits SiHa human cervical cancer cell proliferation through apoptotic induction. It was found that the results of one study suggest an association between p38 β not p38 α and oral cancer development. Additionally, the anti cancer activity of thymoquinone (TQ) indicated that the antitumor activity of TQ may be attributed to the downregulation of p38 β MAPK.⁵

N. sativa can efficiently reduce the number of cells with NF200 at all the CA1, CA2, and CA3 regions of hippocampus and parietal and frontal areas of the rat's brain; *N. sativa* can be used as a potential preventive supplement compound for the treatment of Alzheimer's disease.⁶ It is also used as an antitumor agent,^{7,8} diuretic agent,⁹ analgesic agent,¹⁰ anti-cestode and anti-nematode agent,¹¹ antidiabetic agent,^{12,13} bactericide,¹⁴ and

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antiulcerogenic agent.¹⁵ The antioxidant effect of *N. sativa* oil and its components may represent another mechanism that contributes to its antimalarial activity.¹⁶

The desirable effect of *N. sativa* on human diseases may be due to the presence of active compounds (ie, polyunsaturated fatty acids, TQ, phenolic compounds, and other natural antioxidants) in *N. sativa* oil composition. *N. sativa* seed contains 26.6% oil, of which the major fatty acids are linoleic acid (LA, 64.6%) and palmitic acid (20.4%) as reported in a previous study.¹⁷ Cheikh-Rouhou et al¹⁸ also found that LA was the major unsaturated fatty acid in *N. sativa* (50.3% “Tunisian NS” and 49.2% “Iranian NS”). It was found that *N. sativa* seeds contain 36%–38% fixed oils, proteins, alkaloids, saponin, and 0.4%–2.5% essential oil, and it was reported that the *N. sativa* essential oil is mainly composed of TQ (27.8%–57.0%), ρ -cymene (7.1%–15.5%), carvacrol (5.8%–11.6%), t-anethole (0.25%–2.3%), 4-terpineol (2%–6.6%), and longifolene (1.0%–8.0%).¹⁹ Fatty acid profiles were identified for *N. sativa* seed oils. Also LA and oleic acids are the main fatty acids of *N. sativa* essential oil. In addition, the *N. sativa* essential oil contains considerable amounts of myristic acid (C14:0), arachidic acid (C20:0), and behenic acid (C22:0).²⁰

Most properties of whole seeds or their extracts are mainly attributed to quinine constituents, TQ is the most abundant compound.²¹ TQ, as the main constituent of the volatile oil from *N. sativa* seeds, has been reported to possess strong antioxidant properties.²² Based on a previous study, TQ, carvacrol, t-anethole, and 4-terpineol were identified as active antioxidants and radical scavenging compounds in *N. sativa*.²³ TQ can be used for the treatment of fungal skin infections.²⁴ Previous researchers reported different contents of TQ that ranged from 27.8% to 57% in several Australian *N. sativa* oils.²³

N. sativa oil is traditionally obtained by mechanical pressing or treating powdered *N. sativa* seed with organic solvents, most commonly hexane. Conventional solvent extraction techniques produce low-quality oil that requires extensive purification operations resulting in thermal degradation and loss of valuable compounds.²⁵ The use of organic solvents and the necessity of recovering the solvent have led to a move toward cleaner extraction methods, such as supercritical fluid extraction (SFE). SFE is becoming an efficient and worldwide used technique to obtain valuable natural substances from complex materials. SFE-CO₂ has been utilized as a nontoxic alternative to hexane and other organic solvents for the extraction of oil from corn germ,²⁶ cottonseed,²⁷ rapeseed,²⁸ and other oil-bearing materials.²⁹ Thus, the main objective of this study was to compare the oil extraction efficiency and essential oil composition of Iranian and Indian *N. sativa* L. oil extracted by SFE and solvent extraction techniques.

Materials and methods

Chemicals and materials, such as *N. sativa* seed (black cummin seed), were purchased from local markets in Iran and India. The pure standard of TQ (66%) and LA (60%) were provided by Aldrich Chemical Company (Milwaukee, WI, USA). High-performance liquid chromatography grade methanol and n-hexane were supplied by Fisher Scientific (Pittsburgh, PA, USA).

Collection and storage of *N. sativa* seeds

N. sativa L. seeds were cleaned under running tap water, rinsed with distilled water, and air-dried in oven at 40°C overnight. The dried seeds then were ground into powder and sieved through a 250 μ m filter. The samples were kept frozen in amber flasks until analyzed.

Supercritical fluid extraction

In this study, 150 g of the powdered sample was subjected into SFE equipment (SFE-1000F, Thar US Technology, Pittsburgh, PA, USA) at extraction temperature of 40°C under 60 MPa. SFE flow rate was maintained at 20 mL/min using a variable flow restrictor. The yield was collected within 3 hours. The collected pressure and temperature were 0.1 MPa and 25°C, respectively. The extraction was carried out with pure CO₂.

Solvent methods

N. sativa powder of 10 g was measured by mean of digital scale (Shimadzu, Tokyo, Japan) and placed in flask. A total of 50 mL of solvent was added to each flask. The top of the flasks was covered by aluminum foil. The flasks were shaken using Shaking Incubator (Heidolph Unimax 1010, Heidolph Instruments GmbH & Co. KG Walpersdorfer, Schwabach, Germany) at 5–7 rpm for 4 hours. The extracts were filtered by One Man paper (no 1) twice. The extracts were evaporated by means of rotatory evaporator (Heidolph Laborota 4000, Germany). The yields were measured by digital scale (Shimadzu) and protected from light by aluminum foil. The oils were kept in a refrigerator (–80°C) for further analysis.

Gas chromatography-mass spectrometry

Identification of fatty acid composition of extracted *N. sativa* oil was performed by means of a gas chromatography (Thermo Finnigan TRACE GC-Polaris Q, San Jose, CA, USA) equipped with mass spectrometry detector (Thermo Finnigan) and DB-Wax column (60 m \times 0.25 mm id \times 0.25 μ m film thickness; Supleco, MA, USA). Helium was used as a carrier gas (1 mL/min). Oven temperature was programmed at 70°C for 5 minutes, then raised up to 230°C at 2°C/min

Table 1 The essential oil composition of Iranian and Indian *Nigella sativa* L. seed oils identified using GC-MS

Compound	SFE, %		Hex, %		Met, %		Hex/Met, %	
	Ir	In	Ir	In	Ir	In	Ir	In
ρ -cymene ^{a,b}	8.19	0.54	10.43	1.08	–	–	3.64	2.05
α -thujene ^b	0.86	–	–	–	0.12	–	0.93	–
Thymoquinone ^{a,b}	8.26	0.72	17.54	1.16	21.03	16.0	13.52	8.65
Carvacrol ^{a,b}	1.65	–	2.63	–	3.67	4.83	1.98	1.34
Myristic acid ^{b,c}	0.34	0.29	–	0.26	–	–	–	–
Ascorbic acid	8.07	6.81	–	15.63	5.31	5.86	6.93	7.73
Oleic acid ^{b,c}	2.86	18.97	1.64	19.54	6.54	2.85	5.99	4.53
Linoleic acid ^{b,c}	49.43	61.85	31.91	58.78	22.14	31.9	32.37	58.04
4-terpineol ^{b,c}	0.17	–	–	–	0.35	0.41	–	–
Camphor ^a	–	0.08	1.42	–	–	0.44	–	3.59
<i>t</i> -butylhydroquinone	–	0.38	–	–	7.73	11.5	3.1	–
Valencene	–	–	0.51	0.37	–	–	–	–
Acetic acid	–	–	0.71	–	1.47	–	–	–
Decanoic acid	–	–	7.15	–	–	–	–	–
Ledol	–	–	2.92	–	–	–	2.41	–
Palmitic acid ^{b,c}	–	–	–	–	–	–	1.44	1.34

Notes: ^aBurits et al.;²³ ^bNickavar et al.;³¹ ^cÜstun et al.²⁰

Abbreviations: GC-MS, gas chromatography-mass spectrometry; Hex, hexane; In, Indian; Ir, Iranian; Met, methanol; SFE, supercritical fluid extraction.

and held for 10 minutes at 230°C. Injector temperature was 230°C. EI mass spectra was recorded at 70 eV ionization voltage over the mass range 40–400 u. For gas chromatography-mass spectrometry (GC-MS) analysis, 0.5 μ L of extract and mixture solvent (1:10) containing n-hexane and methanol (1:1 v/v) was injected under split mode (1:33).

Results and discussion

The chemical compositions of *N. sativa* seed identified by GC-MS are listed in Table 1. The gas chromatographs obtained

from GC-MS analyses of oil extracted using solvent and SFE are shown in Figures 1–4. In total, 41 constituents were identified in the essential oils obtained from Iranian and Indian *N. sativa* seeds by using SFE and solvent extraction techniques.

In general, the oil extraction efficiency obtained from SFE was higher than the extracted yield achieved by solvent extraction technique. Among the main compounds identified by GC-MS, LA (22.4%–61.85%) was the main fatty acid in the *N. sativa* oils extracted by both SFE and solvent extraction techniques. Besides LA, oleic acid (1.64%–18.97%),

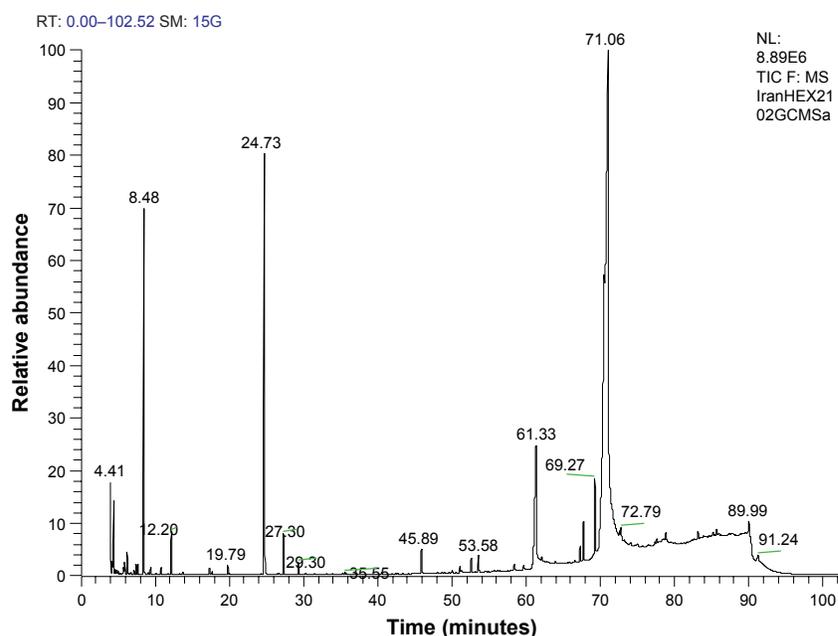


Figure 1 Gas chromatogram of Iranian *Nigella sativa* L. oil extracted by using hexane.

Abbreviations: GC-MS, gas chromatography-mass spectrometry; RT, room temperature; SM, solvent micro; NL, nonpolar lipids; TIC F, total ion current focusing; MS, mass spectrometry.

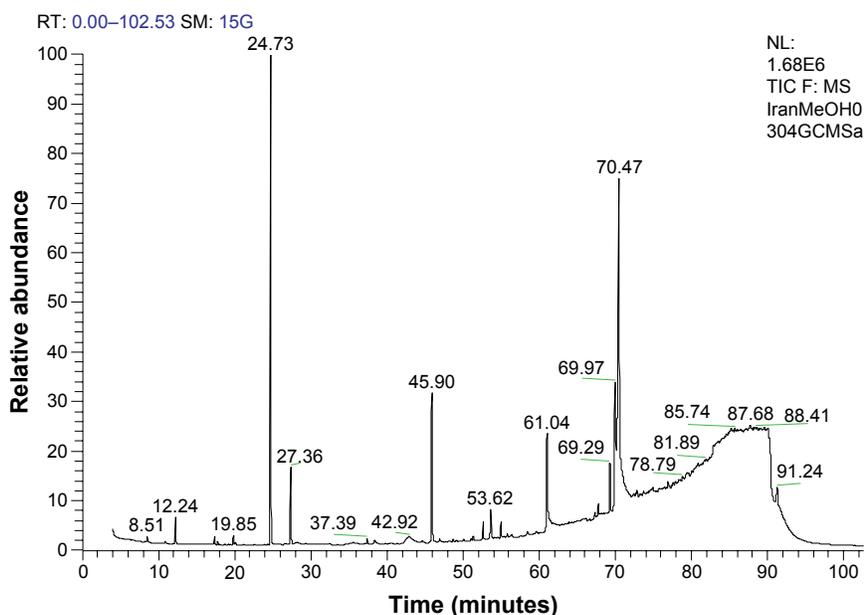


Figure 2 Gas chromatogram of Iranian *Nigella sativa* L. oil extracted by using methanol.

Abbreviations: GC-MS, gas chromatography-mass spectrometry; RT, room temperature; SM, solvent micro; NL, nonpolar lipids; TIC F, total ion current focusing; MS, mass spectrometry.

decanoic acid (7.15%), eicosadienoic acid (2.56%), palmitic acid (1.34%–1.44%), propanoic acid (0.79%), acetic acid (0.71%), heneicosanedioic acid (0.65%), and myristic acid (0.29%–0.34%) were found to be the major compounds identified in essential oils obtained from the Iranian and Indian *N. sativa* seed.

TQ was the main volatile oil component identified in this study (0.72%–21.03%). Some noticeable compounds such as *t*-butylhydroquinone (0.3%–11.5%), carvacrol

(1.34%–4.83%), α -phellandrene (1.66%), longifolene (0.06%), pseudopelletierine (0.15%), neolignan (0.01%), eremophilene (0.18%), methyl palmitoleate (0.65%), limonene (0.32%),^{1,2} cuparene (0.1%), arsaol (0.43%), 2,3-epoxycarane, (E)-4-isopropyl-5-methylhexa-2,4-dien-1-ol (1.05%), 3-cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl (0.4%), tetraacetyl-d-xylonic nitrile (0.1%), thujol (0.12%), ocimene (0.09%), cis-p-mentha-2,8-dien-1-ol (0.32%), benzenemethanol, $\alpha,\alpha,4$ -trimethyl (0.15%), ethyl iso-allocholate (0.08%),

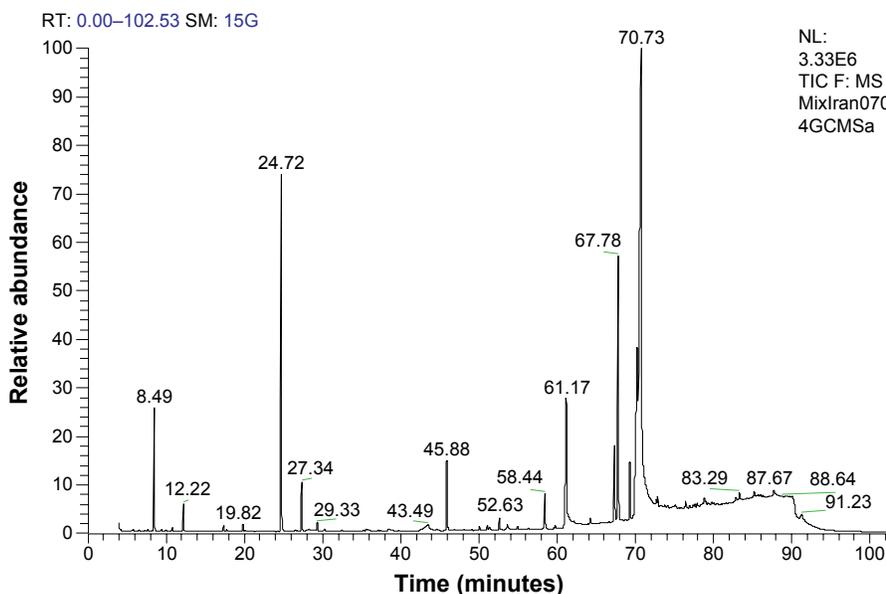


Figure 3 Gas chromatogram of Iranian *Nigella sativa* L. oil extracted by using methanol/hexane.

Abbreviations: GC-MS, gas chromatography-mass spectrometry; RT, room temperature; SM, solvent micro; NL, nonpolar lipids; TIC F, total ion current focusing; MS, mass spectrometry.

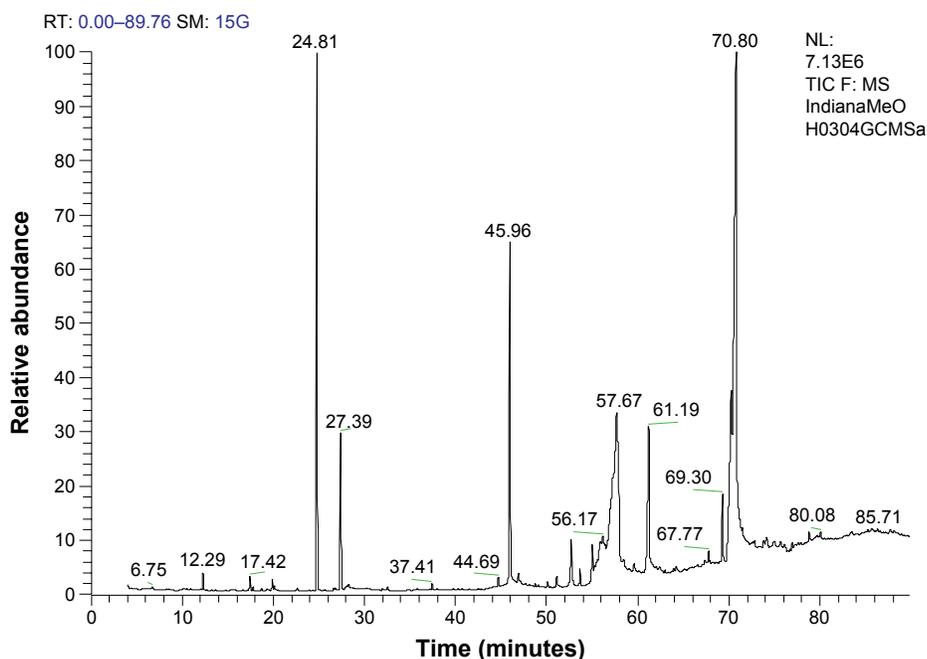


Figure 4 Gas chromatogram of Indian *Nigella sativa* L. oil extracted by using methanol.

Abbreviations: GC-MS, gas chromatography-mass spectrometry; RT, room temperature; SM, solvent micro; NL, nonpolar lipids; TIC F, total ion current focusing; MS, mass spectrometry.

benzeneacetonitrile, 4-hydroxy (0.33%), 1-heptatriacontanol (2.06%), and butylated hydroxytoluene¹ (0.49%) were identified in essential oil obtained from Iranian and Indian *N. sativa* seeds.

The results indicate that the LA of Indian *N. sativa* oil extracted by SFE method was higher than the fatty acid contents measured for the other samples; while the essential oil extracted from Iranian *sativa* seeds using solvent extraction technique (methanol) contained the highest TQ content (21.03%) compared to TQ recorded in the other samples. The present results are in agreement with the results of previous researchers^{21,23} who pointed out that the main compounds in the *N. sativa* essential oil were TQ, carvacrol, ρ -cymene, and 4-terpineol. Regarding the extraction efficiency obtained by using SFE, this study is in accordance with Kao et al,³⁰ who indicated that the oil extraction using SFE resulted in higher content of compounds compared to solvent extraction technique.

Conclusion

The present study shows that SFE and solvent extraction methods have both advantages and disadvantages for the extraction of the essential oil and volatile oil components. Based on the results, SFE has been proven to be an efficient method of extraction. Solvent extraction can be utilized in extraction of some compounds, such as TQ. The present study also demonstrated that SFE is more efficient for extraction of

essential oil from *N. sativa* L. seed compared to the solvent extraction technique. The essential oil obtained from SFE process contained more LA than that achieved by solvent extraction. It was observed that the oil extraction efficiency obtained from SFE is higher than that achieved by solvent extraction technique. According to this research, *N. sativa* oil has some bioactive compounds such as TQ and LA.

Disclosure

The authors report no conflicts of interest in this work.

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