Phytochemical analysis and Anticancer Potency of Black Cumin Oil (*Nigella sativa Linn.*) Against Osteosarcoma

Raad A Kaskoos [1], Javed Ahamad [2 *]

Abstract

Background: Black cumin (*Nigella sativa Linn.*) is a widely accepted remedy and spice in Middle Eastern countries. Traditionally, it has been used for treating and preventing various chronic diseases, including cancer, diabetes, and respiratory conditions. Methods: This study aimed to characterize the chemical components of black cumin oil using gas chromatography-mass spectrometry (GC-MS) and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. Additionally, the anticancer activity of black cumin oil was evaluated using an MTT assay on MG-63 human osteosarcoma cell lines. Results: The GC-MS analysis identified 30 chemical compounds in black cumin oil. The major non-volatile components were thymohydroquinone (31.40%), linoleic acid (18.23%), stearic acid (18.02%), 2,4-decadienal (E,E) (11.14%), diethyl phthalate (5.22%), and palmitic acid (4.33%). The major volatile components included citronellal (0.29%), carvacrol (0.35%), β-pinene (0.41%), and limonene (0.19%). FTIR analysis confirmed the presence of alkanes (hydrocarbons), aldehydes, aliphatic esters, cyclopentanone, and alkenes. The oil exhibited dose-dependent (0.5-500 μg/mL) and time-dependent (24 h) inhibition of MG-63 cell lines, with an IC50 value of 33.66 μg/mL. Conclusion: This study enhances the understanding of the chemical composition of black cumin oil and provides evidence supporting its ethno-medicinal use by demonstrating its anticancer activity.

Keywords: ATR-FTIR, Black cumin, Cancer, GC-MS, MG-63 cell lines, Nigella sativa.

Significance | Black cumin oil's rich chemical composition and significant anticancer activity against osteosarcoma cells support its traditional medicinal uses.

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1. Introduction

Nigella sativa L. (Family: Ranunculaceae) commonly known as black cumin, and it is considered a very important medicine for the treatment of human ailments because of its presence in history and religious texts (Ahmad et al., 2013; Kooti et al., 2016). Black cumin is also known as *Tibb-e-Nabwi* (Prophetic Medicine) and is known to cure all human ailments except death (Ijaz et al., 2017). *N. sativa* is an annual flowering plant, and it has been cultivated in the Indo-pacific region for use in traditional medicine and as a condiment for a long time (Padhye et al., 2008). Black cumin seeds are used in a variety of baked foods, as well as pickles, cuisines, and traditional recipes (Hassanien et al., 2015; Ramadan, 2007). Black cumin oil, extracts, and its bioactive compound thymoquinone were used in the treatment of diabetes (Heshmati et al., 2015), cancer (Mostofa et al., 2017; Randhawa and Alghamdi, 2011; Yi et al., 2008), arthritis (Vaillancourt et al., 2011), and asthma (Boskabady et al., 2010). It is also reported as a potent antioxidant (Ardiana et al., 2020), anti-inflammatory (Alemi et al., 2013; Woo et al., 2012), antimicrobial an immunomodulatory agent (Majdalawieh and Fayyad, 2015) and histology analysis (Hussein et al. 2024).

The chemical composition of *N. sativa* seed oil was studied by

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several authors, and several volatile components have been identified such as thymoquinone, thymohydroquinone, dithymoquinone, *p*-cymene, α-thujene, γ-terpinene, carvacrol, α-pinene, β-pinene, 4-terpineol, and sesquiterpene longifolene, carvone, limonene, and citronellol (Rchid et al., 2004; Wajs et al., 2008; Hamrouni-Sellami et al., 2008). However, several unknown volatile and non-volatile components must be identified depending on the place where the seeds were grown. In humans, many of these phytochemicals have pharmacological effects and therapeutic promise including thymoquinone (Gholamnezhad et al., 2016). To the best of our knowledge, the chemical composition of black cumin seed oil found in Kurdistan region of Iraq was not studied and not assessed for anticancer activity. Hence, the objective of the present study is to characterize the chemical composition of black cumin seed oil by GC-MS and ATR-FTIR methods and determine its anticancer activity in an *in-vitro* model using MG-63 cell lines.

2. Materials and Methods

2.1. Plant Materials and Chemical

The dried seeds of *Nigella sativa Linn.* (1.5 Kg) were collected from the local market of Erbil, Iraq in the month of May 2021. The authenticity of accession was done by Dr. Raad A Kaskoos, Faculty of Pharmacy, Hawler Medical University, Erbil, Iraq. The authenticated plant sample was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Erbil, Iraq for future reference (voucher number: PRL/2021/15). MG-63 (Human osteosarcoma) cell lines were purchased from NCCS, Pune, India. DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/mL) were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plates and wash beakers were from Tarson (India).

2.2. Isolation of N. sativa Oil

The dried seeds of *N. sativa* (1 Kg) were cleaned and milled using a laboratory-type hydraulic press using a pressure of 3 kg cm^{-2} for 15 min to obtain the oil. Then oil was filtered through a glass funnel plugged with cotton. The isolated oil was stored at 4 °C in a Refrigerator for further use.

2.3. GC-MS Analysis and Identification of Chemical Compounds in N. sativa Oil

The aroma and fixed oil composition of *N. sativa* seed oil were determined by the GC-MS. The sample was run on Agilent Bench Top GC-MS (Agilent Technologies, Wilmington, DE, USA) equipment, and it was fitted with a capillary column of DB-5 glass (30 m × 0.25 mm i.d.; film thickness of 0.25 µm). Helium was used at a flow rate of 1 mL/min as the carrier gas. The temperature of the oven was set to 50 °C for 1 min and then isothermally kept for 2 min at 320 °C, while the injector port was maintained at 280 °C. The *N. sativa* seed oil (0.1 µL, in hexane) was injected and the split ratio was kept at 1:5. Data capture took place at 70 eV using scanning times of 1.5 sec in the mass range of 50-1000 amu and run time was kept upto 37 min. The chromatography and mass spectra were handled with Chem station software (Agilent Technologies, Wilmington, DE, USA).

The individual peaks/constituents were identified by comparison of their Kovats Index (K.I.) with those of the literature. Further identification of chemical constituents was made by comparison of the fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NIST, NBS 54 K.L, WILEY8 libraries, and published literature (Adams, 2007; Ali, 2001; Kabiret et al., 2020; Gerige et al., 2009). The percent composition of the extract compounds was calculated based on the area of respective peaks.

2.4. ATR-FTIR Study of N. sativa Oil

The chemical composition of *N. sativa* oil was further analyzed by ATR-FTIR (attenuated total reflectance-Fourier transform infrared). ATR-FTIR (IRAffinity-1S, Shimadzu, Japan) was utilized to record IR spectra of *N. sativa* oil. The spectra acquisition was performed in the spectral range of 400 to 4000 cm^{-1}. Black cumin oil sample was placed on the ATR surface and FTIR spectra were recorded using 45 scans and 4 cm^{-1} resolutions. The ATR surface was cleaned using ethanol and a background scan was recorded before each sample scan.

2.5. Anticancer Activity of N. sativa Oil

The *N. sativa* seed oil was tested for *in-vitro* cytotoxicity, using MG-63 cells by MTT assay. The assay method was performed as per the method described by Marquez et al. (2020). Briefly, the MG-63 cell lines were cultured in liquid medium (DMEM) supplemented with 10% FBS, 100 ug/mL penicillin, and 100 µg/mL streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C. The cultured MG-63 cells were harvested by trypsinization and then pooled in a 15 mL tube. The cells were plated at a density of 1×10^5 cells/mL cells/well (200 µL) into the 96-well plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24 to 48 hours at 37°C. The cells were washed with sterile PBS and treated with *N. sativa* oil (conc. 0.5 to 500 µg/mL) in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/mL) was added into each well and the cells were incubated for a further 2-4 h until purple precipitates were visible under an inverted microscope. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µL). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added, and the plate was shaken for 5 min. The absorbance was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell

viability and IC_{50} value was calculated using Graph Pad Prism 6.0 software (USA).

3. Results and Discussion

3.1. GC-MS Analysis of N. sativa Oil

The black cumin oil was isolated by a hydraulic press machine, and it gave brownish color oil (yield 10.46±1.06% *v/w*). Black cumin oil was analyzed by GC-MS, and the results are presented in Table 1 and Figure 1. The GC-MS analysis yielded thirty chemical compounds which constitute about 99.98% of total oil. Thymohydroquinone (31.40%), linoleic acid (18.23%), stearic acid (18.02%), 2,4-decadienal, (*E,E*) (11.14%), diethyl phthalate (5.22%), palmitic acid (4.33%), methyl palmitate (2.81%), stearyl alcohol (1.12%), and methyl linoleate (1.35%) were found as major chemical compounds in black cumin oil. Other important chemical compounds identified in black cumin oil were *m*-cymene (0.97%), thymoquinone (0.19%), hexadecanal (0.38%), citronellal (0.29%), carvacrol (0.35%), *β*-pinene (0.41%), and limonene (0.19%) (Table 1).

Black cumin is known for its ethnopharmacological use in the treatment of diabetes, asthma, arthritis, cancer, and digestive problems (1,3). The present explores the chemical composition of black cumin oil by GC-MS method. The GC-MS analysis resulted identification and characterization of 30 chemical compounds in which thymoquinone derivative as thymohydroquinone (31.40%) was found as a major compound. Several authors reported that black cumin's bioactivities are due to thymoquinone and other related compounds (8-11,15,16). The other chemical compounds such linoleic acid (18.23%), stearic acid (18.02%), 2,4-decadienal, (*E,E*) (11.14%), diethyl phthalate (5.22%), palmitic acid (4.33%), and methyl palmitate (2.81%) were found as major compounds which are in close agreement with previous studies (Rchid et al., 2004; Wajs et al., 2008; Kabir et al., 2020).

3.2. ATR-FTIR Analysis of N. sativa Oil

The black cumin oil was further analyzed by ATR-FTIR and the results were presented in Table 2 and Figure 2. FTIR analysis shows the peak values (cm^{-1}) corresponding to functional groups of alkanes, aldehydes, aliphatic esters, cyclopentanone, fluoro compounds, primary alcohol, and alkenes (mono and di-substituted) as evident in Figure 2.

FTIR is one of the most extensively used technologies for the identification of chemical constituents and elucidation of complex chemical structures. In recent years, FTIR has played a significant role in pharmaceutical analysis due to its unique characteristics and broad applicability (Vlachos et al., 2006). Black cumin oil was further analyzed by the ATR-FTIR method for confirmation of different types of chemical compounds. The FTIR analysis shows the presence of alkanes (hydrocarbons), aldehydes, aliphatic esters, cyclopentanone, fluoro compounds, primary alcohol, and alkene (mono and di-substituted) compounds.

3.3. Anticancer Activity of N. sativa Oil

The MTT assay was performed to assess the anticancer activity of black cumin oil using MG-63 cell lines. The *in-vitro* anticancer results are presented in Figure 3 and Figure 4A-G. From Figures 3 and 4B-G, it is evident that black cumin oil produces dose (0.5 to 500 µg/mL) and time (24h) inhibition of MG-63 cell lines. The cell viability ranges from 91.39±8.43 to 21.73±0.76 for concentrations of 0.5 to 500 µg/mL, respectively (Figure 3) after 24 h treatment with black cumin oil. The IC_{50} value was found 33.66 µg/mL for black cumin oil against MG-63 cell lines. As shown in Figure 4A, the untreated MG-63 cells maintained their original morphology and close contact with each other even when the incubation was prolonged to 24 hrs. In contrast, MG-63 cells lost their original shape at 24 hrs after treatment (Figure 4B-G). When the treatment was extended to 48 hrs, suspension cells (dead cells) were identified, and more suspension cells were observed at 24 hrs.

Osteosarcoma is the most common type of tumor found in bone marrow, and it has a poor prognosis and is associated with metastatic disease. The treatment of osteosarcoma includes surgery combined with chemotherapy (Anoop et al., 2014). Natural products are known for their preventive role in cancer (Ahamad et al., 2019; Gezici et al., 2019). Black cumin has been reported as a potential anticancer remedy (Mostofa et al., 2017; Yi et al., 2008). In the present study, black cumin oil anticancer activity was assessed by using MG-63 (Human osteosarcoma) cell lines. Black cumin oil produces dose (0.5-500 µg/mL) and time (24 h) dependent inhibition of MG-63 cell lines with the IC_{50} value of 33.66 µg/mL. As shown in Figure 4, the untreated MG-63 cells maintained their original morphology and close contact with each other even when the incubation was prolonged to 24 hrs. In contrast, MG-63 cells lost their original shape at 24 hrs after treatment. The MG-63 cell lines had lost their elongated spindle-shaped morphology. When the treatment was extended to 48 hrs, suspension cells (dead cells) were identified, and more suspension cells were observed at 24 hrs. The *in-vitro* MTT study results suggest the potential anticancer activity of black cumin oil.

4. Conclusion

The chemical composition of *N. sativa* (black cumin) seed oil was determined by GC-MS and ATR-FTIR methods. In the present study, thymoquinone derivative as thymohydroquinone was found as most predominant component. The *in-vitro* anticancer study shows dose and time-dependent inhibition of MG-63 cell lines. The present study leads identification of phytochemicals and anticancer potential of black cumin seed oil, assisting in therapeutic claims regarding this species in the traditional system.

Table 1. Volatile and non-volatile components of black cumin oil (*N. sativa*)

S. No.	Name of chemical compounds	RT	RI	% Composition
1.	2-Heptenal, (*E*)	6.927	930	0.33
2.	*α*-Pinene	7.097	937	0.12
3.	*β*-Pinene	7.637	979	0.41
4.	*α*-Phellandrene	8.098	1005	0.11
5.	*m*-Cymene	9.206	1022	0.97
6.	Limonene	11.316	1023	0.19
7.	*α*-Thujene	14.163	1026	0.20
8.	*γ*-Terpinene	14.288	1059	0.24
9.	Thymoquinone	14.957	1249	0.19
10.	*(E)*-2-Decenal	15.419	1263	0.27
11.	Thymol	17.304	1270	0.06
12.	Carvacrol	20.053	1282	0.35
13.	*trans*-Anethole	20.491	1283	0.37
14.	2,4-Decadienal, (*E,Z*)	26.072	1296	0.08
15.	2,4-Decadienal, (*E,E*)	26.549	1317	11.14
16.	Longifolene	27.656	1406	0.39
17.	Citronellal	27.838	1475	0.29
18.	Thymohydroquinone	29.196	1522	31.40
19.	Diethyl Phthalate	29.676	1603	5.22
20.	Hexadecanal	30.324	1819	0.38
21.	6,11-Hexadecadien-1-ol	30.518	1857	0.36
22.	Methyl hexadecanoate	31.840	1909	0.52
23.	Methyl palmitate	32.369	1927	2.81
24.	Hexadecanoic acid (palmitic acid)	32.893	1968	4.33
25.	Stearyl alcohol	33.800	2070	1.12
26.	Methyl linoleate	34.179	2091	1.35
27.	Linoleic acid	35.053	2105	18.23
28.	Octadecanoic acid (stearic acid)	35.082	2158	18.02
29.	Octacosane	36.495	2800	0.42
30.	Oleic acid	36.720	3200	0.11

where, KI: Kovats index, and RT: retention time

Table 2. FTIR results of *N. sativa* seed oil

S. No.	Peak values (cm^{-1})	Bonds	Functional groups
1.	3000.02	C-H stretching	Alkane
2.	2922.16	C-H stretching	Alkane
3.	2852.72	C-H stretching	Aldehyde
4.	1739.79	C=O stretching	Cyclopentanone
5.	1454.33	C-H bending	Alkane (methyl)
6.	1377.17	C-H bending	Aldehyde
7.	1226.73	C-F stretching	Fluoro compound
8.	1163.86	C-O stretching	Aliphatic esters
9.	1028.06	C-O stretching	Primary alcohol
10.	977.91	C=C bending	Alkene (monosubstituted)
11.	912.33	=CH bending	Alkene
12.	717.52	C=C bending	Alkene (disubstituted, cis))

Author contributions

R.A.K. and J.A. conceptualized the study, developed the methodology, and created the software. All auhtors analyzed the data, conducted the investigation. Both wrote the original draft, reviewed and edited the manuscript, and created visualizations. All authors supervised the project, administered the project, and acquired funding.

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Competing financial interests

The authors have no conflict of interest.

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