



UNIVERSITI PUTRA MALAYSIA

**COMPARISON OF CHEMICAL PROFILES OF BLACK CUMIN SEED
(*NIGELLA SATIVA* .L) EXTRACTS AND EVALUATION OF THEIR
CYTOTOXIC EFFECT ON BREAST CANCER MCF-7 AND MDA-MB-
231 CELL LINES**

**KOUROSH HASANZADEH GHAHRAMANLOO
IB 2009 15**

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.L) Extracts and Evaluation of Their Cytotoxic Effect on Breast Cancer
MCF-7 and MDA-MB-231 Cell Lines**

By

Kourosgh Hasanzadeh Ghahramanloo

**Thesis Submitted to the School of Graduate Studies, University Putra
Malaysia, in Fulfilment of the Requirements for the Degree of
Master of Science**

October 2009



Abstract of thesis presented to the Senate of University Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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October 2009

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Breast cancer is one of the main life-threatening diseases that a woman may have to face during her lifetime. This study was proposed in order to investigate the anti-cancer effects of the active ingredients of *Nigella sativa* crude extract, Thymoquinone (TQ), and Linoleic Acid (LA) on breast cancer MCF-7 and MDA-MB-231 cell lines. According to study design of this research, the study was performed in two parts respectively:

1. Extraction and Identification of *Nigella sativa* composition

The main objective of this part of the present study was to compare the extract and oil composition of Iranian and Indian *Nigella sativa* L. extracted, using Super



Critical Fluid Extraction (SFE) and solvent extraction. In this study, Gas Chromatography (GC) equipped with Mass Spectrophotometer (MS) detector was employed for qualitative analysis of the essential oil composition of the samples. The results indicated that the main fatty acids identified in the essential oils and extracted by using SFE and solvent extraction were linoleic acid (22.4-61.85) and oleic acid (1.64-18.97). As shown in results, thymoquinone (0.72-21.03) was found to be the major volatile compound in the extracted *Nigella sativa* oil. The oil extraction efficiency obtained from SFE was shown to be significantly higher than that achieved by solvent extraction technique, qualitatively and quantitatively. The present study showed that SFE can be used as a more efficient technique for extraction of *Nigella sativa* L. seed oil compared with solvent extraction technique.

2. Cell line and Culture. Cell lines were maintained in DMEM supplemented with 10% FBS, penicillin (100 μ /ml), and streptomycin (100 μ g/ml) in a humidified atmosphere of 5% CO₂ at 37 °C. The cytotoxic and apoptotic effects were determined by the MTS assay. The results of MTS assay were supported by flow cytometry (Cell cycle analysis). Also morphological studies using phase contrast were performed by inverted microscope on MCF-7 and MDA-MB-231 cell lines. All experiments were performed for 24, 48, and 72 hours of treatments. The results showed the cytotoxic effect of linoleic acid on the breast cancer cell lines that can be posed as an anticancer effect of linoleic acid. According to our findings, when the concentration of linoleic acid is getting increased, compared with the concentrations currently being reported, it shows anticancer effects. Thymoquinone has a great significant cytotoxic and apoptotic effects on cells. Also the oils of Iranian and Indian samples were shown cytotoxic effect on both of cell lines. The cytotoxic



effect was observed in lower concentrations on MCF-7 to compare with MDA-MB-231. In general, MCF-7 was more sensitive compared with MDA-MB-231. In conclusion, it can be mentioned that linoleic acid and thymoquinone as two major components of oil of *Nigella sativa* have shown strong cytotoxic and apoptotic effects on MDA-MB-231 and MCF-7 breast cancer cell lines. Also the crude extract oil showed inhibiting and apoptotic effects on cancer cells in higher concentration compared with the linoleic acid and thymoquinone.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

**Perbandingan profil kimia daripada ekstrak Bijian Jintan Hitam (*Nigella sativa*
.L), dan penilaian kesan sitotoksiknya pada sel kanser payudara MCF-7 dan
MDA-MB-231**

Oleh

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Kanser payudara merupakan salah satu penyakit pengancam nyawa yang mungkin dihadapi oleh wanita semasa hayat mereka. Kajian ini telah dijalankan untuk mengesan kesan anti kanser bahan aktif Jintan Hitam (*Nigella sativa* .L), terhadap sel kanser payudara MCF-7 dan MDA-MB-231. Kajian ini telah dijalankan dalam dua peringkat seperti berikut:

1. Pengekstrakan dan penentuan komposisi utama minyak mentah

Nigella sativa :

Objektif utama kajian pada peringkat ini adalah untuk membandingkan komposisi minyak Jintan Hitam dari Iran dan India dengan menggunakan kaedah *Super Critical Fluid Extraction (SFE)* dan ekstraksi pelarut (*Solvent Extraction*). Dalam kajian ini, *Gas Chromatography (GC)* yang dilengkapi dengan pengesan *Mass*

Spectrophotometer (MS) di gunakan untuk analisis kualitatif bagi mengecam komposisi minyak utama sampel-sampel *Nigella sativa*. Keputusan kajian menunjukkan kandungan asid lemak utama dalam minyak dikenalpasti dengan menggunakan kaedah SFE dan ekstraksi pelarut adalah asid linoleik (22.4 - 61.85%) dan asid Oleik (1.64 - 18.97%). Keputusan juga menunjukkan thymoquinone (0.72 - 21.03%) adalah kompaun utama dalam minyak mudah meruap dengan kadar tertinggi yang di kesan dalam minyak mentah *Nigella sativa L*. Kandungan ekstrak minyak yang diperolehi melalui kaedah dari SFE menunjukkan kadar yang lebih tinggi berbanding ekstrak yang di perolehi melalui kaedah ekstraksi pelarut secara kualitatif dan kuantitatif. Kajian ini menunjukkan kaedah SFE boleh digunakan sebagai satu teknik lebih efisien untuk pengekstrakan pati minyak *Nigella sativa L* berbanding dengan teknik ekstraksi pelarut (Solvent Extraction Technique).

2. Sel kanser dan kultur: Sel kanser payudara jenis MCF-7 dan MDA-MB-231 di eram dan dipertahankan dalam larutan DMEM yang ditambahkan 10% FBS, penisilin (100 μ /ml), dan ubat antibiotik streptomycin (100 μ g / ml) dalam persekitaran yang lembab dengan 5% gas karbon dioksida (CO₂) pada suhu 37 °C. Kesan-kesan sitotoksik dan kematian sel (apoptotic) telah dikenalpasti melalui cerakin *MTS*. Keputusan bagi cerakinan *MTS* pula telah disokong oleh kaedah sitometri aliran (analisis kitaran sel). Kajian ini juga di lengkapkan dengan pengukuran morfologi sel dengan menggunakan mikroskop arca terbalik berfasa kontras keatas sel kanser payudara MCF-7 dan MDA-MB-231. Semua ujikaji dijalankan selepas 24, 48, dan 72 jam rawatan dengan asid linoleik, asid oleik dan thymoquinone. Keputusan kajian menunjukkan terdapat kesan sitotoksik bagi asid linoleik pada sel kanser payudara yang boleh memberi kesan sebagai antikanser.

Berdasarkan keputusan kajian juga, apabila kepekatan asid linoleik bertambah, berbanding dengan laporan hasil kajian lalu, didapati wujud kesan anti kanser. Thymoquinone pula didapati mempunyai satu kesan yang signifikan dalam menghalang pertumbuhan sel-sel kanser payudara. Minyak mentah *Nigella sativa* dari sampel-sampel dari Iran dan India juga telah menunjukkan kesan sitotoksik pada kedua-dua jenis sel kanser payudara. Kesan sitotoksik pada sel kanser MCF-7 telah dapat diperhatikan dalam kepekatan asid linoleik yang lebih rendah berbanding dengan sel MDA-MB-231. Secara umum kajian ini mendapati sel kanser payudara MCF-7 adalah lebih peka terhadap kesan sitotoksik asid linoleik dan thymoquinone berbanding sel kanser payudara MDA-MB-231. Sebagai kesimpulan, asid linoleik dan thymoquinone sebagai dua komponen utama minyak pati Jintan Hitam (*Nigella sativa*) telah menunjukkan dengan jelas kesan sitotoksik dan kesan-kesan kematian sel (apoptotic) terhadap sel kanser payudara MDA-MB-231 dan MCF-7. Selain itu, kajian juga mendapati ekstrak minyak mentah *Nigella sativa* dapat menghalang pertumbuhan sel-sel kanser payudara dan menunjukkan kesan kematian sel kanser (apoptotic) dalam kepekatan tinggi berbanding dengan asid linoleik dan thymoquinone.

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My gratitude to the staff of IBS and FPSK, UPM for their time and effort spent to participate in this research project as well as my fellow course-mates who helped when I encountered difficulties.

Last but not least, I would like to convey my greatest and deepest thanks and appreciation to my family for their love, care and financial support throughout the period of conducting my research project.



APPROVAL

I certify that the Examination Committee has met on 6th October 2009 and conducted the final examination of Kourosh Hasanzadeh Ghahramanloo on his Master of Science Thesis entitled “**Comparison of chemical profiles of Black Cumin Seed (*Nigella sativa* .L) seed extractions obtained via solvent extraction and Supercritical Fluid Extraction (SFE) techniques and evaluation of their cytotoxic effect on Breast Cancer MCF-7 and MDA-MB-231 Cell lines**” in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and University Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not currently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

KOUROSH HASANZADEH GHAHRAMANLOO

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LIST OF ABBREVIATIONS

1. UPM	Universiti Putra Malaysia
2. WHO	World Health Organization
3. FPSK	Fakulti Perubatan dan Sains Kesihatan
4. <i>N. sativa</i>	<i>Nigella sativa</i> L.
5. NSO	<i>Nigella sativa</i> oil
5. SFE	Super critical Fluid Extraction
6. SE	Solvent Extraction
7. TQ	Thymoquinone
8. LA	Linoleic Acid
9. GC	Gas Chromatography
10. MS	Mass Spectrophotometer
11. GC/MS	Gas Chromatography Mass Spectrophotometer
12. UV	Ultra Violet
13. UKM	Universiti Kebangsaan Malaysia
14. USA/US	United State of America
15. SPSS	Statistical Package for Social Sciences
16. Ir	Iran
17. In	Indian
18. Hex	N-Hexane
19. Met	Methanol
20. IBS	Institute of Bioscience
21. FMHS	Faculty of Medicine and Health Sciences
20. IC ₅₀	50% Inhibitory Concentration
21. TQLARF	TQ and LA rich extract (TQLARE)
22. FFA	Free Fatty Acid
23. CLA	Conjugated Linoleic Acid



CHAPTER 1

INTRODUCTION

1. Introduction

Cancer is one of the major causes of death worldwide. It is estimated that 12.8% of the world population die due to cancer. In the year 2000, 5.3 million men and 4.7 million women developed a malignant tumor and 6.2 million died from the disease. The number of new cases is expected to grow by 50% over the next 20 years to reach 15 million by 2020 and there were 1,050,346 cases reported with 372,969 deaths from breast cancer worldwide (Stewart, 2008). Worldwide, breast cancer is the most prevalent cancer in women. Breast cancer is one of the main life-threatening diseases that a woman may have to face during her lifetime. More than 1 million women worldwide and more than 400,000 women die from it (Stewart, 2003). Breast cancer represents 30.4% of all malignancies among women of all ethnic groups in Malaysia in 2002, with a cumulative lifetime risk of 1:19 (Lim, G.C.C., 2003). The Age Standardized Rate (ASR) of female breast cancer is 52.8 per 100,000 populations (Lim, G.C.C., 2003). Latest statistics from the National Cancer Registry (NCR) show that lung cancer is the most common cancer experienced by men in the country, whilst breast cancer is the top cancer in women. Lung cancer accounts for 13.8 per cent of cancer cases among men. For women, breast cancer accounts for 31 per cent of cases (Lim, G.C.C., 2003).

Several lifestyle factors such as weight gain, obesity, fat intake, and level of physical activity are associated with breast cancer risk. Overweight women are most



commonly observed to be at increased risk of postmenopausal breast cancer and at reduced risk of premenopausal breast cancer. Obesity and a high intake of meat, dairy products, fat, and alcohol may increase risk and a high intake of fiber, fruits, vegetables, anti-oxidants, and phytoestrogens may reduce risk (Farah & Begum, 2003).

2. Phytomedicine

Plant products have been used to cure and prevent diseases through history. Natural compounds in plants, fungi, and bacteria have provided lead structures that have been used to design and plan new drugs in the drug development process. The increasing incidence of breast neoplasia reported over the last few decades has led to development of new anticancer drugs, drug combinations, and chemotherapy strategies by scientific exploration of enormous pool of synthetic, biological, and natural products . In light of the continuing needs for effective anti-cancer agents, and the association of fruits and vegetables consumption with reduced cancer risk, edible plants are increasingly being considered as sources of anticancer drugs. There is a large amount of scientific evidence showing that medicinal plants constitute the main source of new pharmaceuticals and healthcare products, including medications for ethno-veterinary medicine. Recently, cancer chemoprevention with strategies using medicinal herbs has been regarded as one of the most visible fields for cancer control. Currently, researches are focused on plant-derived anti-tumor drugs, antibiotics, and drugs active against tropical diseases, contraceptives drugs, anti-inflammatory drugs, kidney protectors, and drug for psychiatric use. Epidemiological studies suggested that antioxidant supplements might reduce the risk of breast cancer recurrence or breast cancer-related mortality, and consuming food and beverages rich

in poly-phenols (e.g., catechins, flavones, and anthocyanines) is associated with a lower incidence of cancers. Experimental investigations demonstrated that many naturally occurring agents and plant extracts have shown antioxidant and anticancer potential in a variety of bioassay systems and animal models, having relevance to human disease (Aziz et al., 2003). *Nigella sativa*, an oriental spice, has long been used as a natural medicine for treatment of many acute as well as chronic conditions. It has been used in the treatment of diabetes, hypertension, and dermatological conditions (Farah & Begum, 2003). TQ is the bioactive constituent of the volatile oil of black seed, which has shown promising anti-cancer effects on animal models. Moreover, its combination with clinically used anticancer drugs has led to improvements in their therapeutic index and prevents non-tumor tissues from sustaining chemotherapy-induced damage (Muhtasib et al., 2006).

This study was proposed in order to investigate the anticancer effects of the active ingredients of *Nigella sativa* crude extracts, thymoquinone, and linoleic acid on estrogen-receptor positive (ER+) MCF-7 and estrogen-receptor negative (ER-) MDA- MB-231 human breast cancer cell lines.

3. Hypothesis

3.1. H₀ (null hypothesis):

The presence of thymoquinone, linoleic acid, and seed oil of *Nigella sativa* seed doesn't have any significant ($P < 0.05$) anticancer or functional effect on the apoptosis or growth of MCF-7 and MDA-MB-231 Cell lines.



3.2. HA (alternative hypothesis):

Thymoquinone, linoleic acid, and seed oil of *Nigella sativa* have a significant ($P < 0.05$) anti-cancer or functional effect on the apoptosis or growth of MCF-7 and MDA-MB-231 Cell lines.

3. Research Objectives

3.1. To determine and quantify the compositions of essential oil of Iranian and Indian *Nigella sativa L* seed extracted by different methods; different solvents such as methanol, n-hexane, methanol/n-hexane and Supercritical Fluid Extractor (SFE).

3.2. To determine the anti-cancer properties of *Nigella sativa* oil and active target components, thymoquinone and linoleic acid on the growth of MCF-7 and MDA-MB-23 human breast cancer cell lines.

3.3. To determine IC_{50} of TQ, LA, and *Nigella sativa* oil on the growth of MCF-7 and MDA-MB-231 human breast cancer cell lines.

CHAPTER 2

Literature Review

1. Black cumin seed (*Nigella sativa* L.)

Nigella sativa L. (Ranunculaceae) with English name Black cumin seed or black seed, is traditionally known in Middle Eastern countries as “Habbat al Barakah”- ‘The Blessed Seed’, due to its powerful healing qualities for many ailments. It has been used for thousands of years in the Middle East as well as parts of Asia and Africa and is now well known in the USA and Europe. The earliest written reference to Black seed is found in the book of Jesus in the Old Testament 28:25-27. Ibn Sina, Qanun of Medicine, one of the most famous books in the history of medicine, recommended *Nigella sativa* L to stimulate the metabolism and removes dispiritedness and lethargy. The Greek Physician Dioskorides used Black seed to treat headaches and nasal congestion, toothache, and intestinal parasites. Hypocrates, the grandfather of today’s scientific medicine regarded Black seed as a valuable remedy in hepatic and digestive disorders. Ayurvedic medicine appreciates its many qualities and bitter, warming, stimulant nature. Here it is used for a wide variety of diseases like hemorrhoids, hepatitis, fever, diarrhea, cough, and tapeworm, to mention only a few of them.

2. Seed oil:

The oil of Black seed is so beneficial due to its content of over a hundred components such as aromatic oils, trace elements, vitamins, and enzymes. It contains 58% of essential fatty acids including omega 6 and omega 3. These are necessary for

