Pharmacogn J. 2025; 17(3): 307-313
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Phytochemical Analysis and Pro-Melanogenic Activity of *Nigella sativa* Extract in B16F10 Cells: A Natural Candidate for Vitiligo Treatment

Dian Pratiwi[1,*], Silmi Mariya[2], Raendi Rayendra[3], Agus Setiyono[4]

ABSTRACT

Background: Vitiligo is a chronic depigmentation disorder caused by the selective destruction of melanocytes, with limited effective treatment options, particularly in resource-limited regions. *Nigella sativa* (black cumin seed, BCS) has traditionally been used for various skin ailments, including pigmentation disorders. **Objectives:** This study aimed to evaluate the pro-melanogenic activity of a 96% ethanol extract of Indonesian BCS (EE BCS) in B16F10 cells. **Methods:** Phytochemical profiling was performed using gas chromatography–mass spectrometry (GC-MS), and thymoquinone (TQ) content was quantified by high-performance liquid chromatography (HPLC). Cell viability (MTT assay), tyrosinase activity (L-DOPA assay), and melanin content were measured. **Results:** GC-MS identified 9,12-octadecadienoic acid methyl ester (E,E) as the predominant compound (45.88%), while HPLC confirmed a relatively low TQ concentration (0.04%). EE BCS maintained >90% cell viability at concentrations up to 12.50 ppm and exhibited an IC50 of 56.41 ppm. Tyrosinase activity significantly increased at 6.25 ppm (136.40%; $p < 0.05$) and 12.50 ppm (228.10%; $p < 0.01$), accompanied by a significant elevation in melanin content (226.00%; $p < 0.05$ and 266.90%; $p < 0.01$ respectively). **Conclusion:** EE BCS can effectively promote melanogenesis, despite containing low levels of thymoquinone, potentially through synergistic actions of its phytochemical constituents. Given its region-specific phytochemical richness, Indonesian *N. sativa* extract holds promise as a natural therapeutic candidate for vitiligo. Further *in-vivo* and clinical validation is warranted.

Key words: B16F10, Melanogenesis, *Nigella sativa,* Tyrosinase, Thymoquinone, Vitiligo.

Dian Pratiwi[1,*], Silmi Mariya[2], Raendi Rayendra[3], Agus Setiyono[4]

[1]*Doctoral Program of Animal Biomedical Sciences, School of Veterinary Medicine and Biomedicine, IPB University, INDONESIA.*

[2]*Primate Animal Study Center, IPB University, INDONESIA.*

[3]*Faculty of Medicine, Syarif Hidayatullah Islamic University, INDONESIA.*

[4]*Pathology Division, School of Veterinary Medicine and Biomedicine, IPB University, INDONESIA.*

Correspondence

Dian Pratiwi

Doctoral Program of Animal Biomedical Sciences, School of Veterinary Medicine and Biomedicine, IPB University, INDONESIA.

Email: dianpratiwi@apps.ipb.ac.id

History

- Submission Date: 01-05-2025;
- Review completed: 27-05-2025;
- Accepted Date: 05-06-2026.

DOI : 10.5530/pj.2025.17.39

Article Available online

http://www.phcogj.com/v17/i3

INTRODUCTION

Vitiligo is a common depigmentation disorder caused by the progressive loss of functional melanocytes, characterized by chalky white macules on the skin, mucosa, and hair. Although not life-threatening, vitiligo can lead to significant psychological distress and social stigma, particularly in populations with darker skin tones. In resource-limited settings such as many regions of Indonesia, access to standard therapies—such as phototherapy, or calcineurin inhibitors—remains inadequate due to cost, availability, and long treatment durations.[1] Therefore, there is a growing interest in alternative, affordable, and sustainable treatments using natural products.

Today, an estimated 80% of people worldwide use traditional herbal medicines, highlighting the relevance of phytotherapy,[2] including in dermatological conditions like vitiligo. Among potential botanical agents, *Nigella sativa* (black cumin), a flowering plant from the Ranunculaceae family, has garnered attention for its broad pharmacological properties[3] and it is also cultivated in Indonesia as an introduced species.[4] Traditionally used across Asia and the Middle East, its seeds and oil have been applied to treat conditions ranging from asthma, diabetes, inflammation, and skin disorders,[3,5] In dermatological applications, the plant has demonstrated potential in promoting wound healing, reducing inflammation, and possibly enhancing skin pigmentation.[6]

The pharmacological activity of *N. sativa* is largely attributed to thymoquinone (TQ), its major bioactive component,[7] which exhibits anti-inflammatory[8], antioxidant, and immunomodulatory effects[9], and anticancer properties.[10] However, other phytoconstituents—such as flavonoids, terpenoids, and phytosterols—may act synergistically with TQ to modulate melanogenesis. As such, whole-extract approaches may provide therapeutic advantages over isolated compounds. To effectively extract this broad range of bioactives, 96% ethanol is frequently used, as it is capable of dissolving both polar and moderately nonpolar compounds, ensuring comprehensive phytochemical recovery.[11]

Despite the global use of *N. sativa*, its therapeutic potential in vitiligo remains understudied in the Indonesian context. *In-vivo* studies from Middle Eastern countries have demonstrated skin repigmentation effects using *N. sativa* oil[12–14], yet differences in different geographical regions and cultivation practices may lead to regional variation in chemical composition and efficacy. Previous GC-MS analysis have shown significant differences between Indonesian-grown and imported BCS.[15] Therefore, it is important to evaluate the melanogenic potential of *N. sativa* cultivated in Indonesia to support locally sourced therapeutic solutions.

Given this background, the study aimed to investigate the pro-melanogenic activity of a 96% ethanol extract of Indonesian black cumin seeds (EE BCS) using the B16F10 mouse melanoma cell line—a well-established *in-vitro* model for melanin production[16], with a focus on tyrosinase activity and melanin content.

Cite this article: Pratiwi D, Mariya S, Rayendra R, Setiyono A. Phytochemical Analysis and Pro-Melanogenic Activity of *Nigella sativa* Extract in B16F10 Cells: A Natural Candidate for Vitiligo Treatment. Pharmacogn J. 2025;17(3): 307-313.

MATERIAL AND METHODS

Chemical and reagents

Reagents for assays included 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 3-isobutyl-1-methylxanthine (IBMX), and thymoquinone standard ≥97% were from Sigma-Aldrich (USA). L-3,4-dihydroxyphenylalanine (L-DOPA) was from Santa Cruz Biotechnology Inc. (USA). Cell culture reagents included Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA), 10% foetal bovine serum (FBS; Hyclone, USA), 1% penicillin-streptomycin (Invitrogen, USA), phosphate-buffered saline (PBS; Gibco, USA), 0.25% trypsin (Gibco, USA), dimethylsulfoxide (DMSO; Sigma, USA). All other reagents used were of analytical grade.

Black cumin seeds extraction

Plant Material

Black cumin seeds were sourced from Karangpandan, Karanganyar Regency, Central Java, Indonesia (7° 36′ 55.6″ S, 111° 3′ 54.38″ E), and authenticated at the Bogoriense Herbarium, Indonesian Institute of Sciences.

Extraction

The extraction was conducted using the maceration method with 96% ethanol, following the procedure outlined by Laksmiani *et al.*[17] Black cumin seed powder was weighed and macerated in ethanol at a powder-to-solvent ratio of 1:10 (w/v). The mixture was stirred occasionally and left for 24 h before filtration to obtain the initial maceration extract. The residue was re-macerated with fresh solvent for another 24 h. The macerated and re-macerated extracts were combined and concentrated using a rotary evaporator. The resulting extract was evaporated in an oven at 40°C and 50 rpm until concentrated and subsequently stored in a dark glass vial at 4°C until analysis.

GC-MS Analysis of Phytochemicals

Phytochemical profiling was conducted using a GC-MS system (Agilent 7890B GC coupled with 5977A MSD). Separation was achieved using a HP-5MS column (30 m × 250 µm × 0.25 µm) with helium as the carrier gas at 1 mL/min. The oven temperature was initially set to 50°C for 5 min, then increased to 280°C at a rate of 5°C/min and held for 20 min, with a total run time of 71 min. The MS was operated in electron impact mode at 70 eV, scanning from 33 to 600 m/z. The ion source temperature was set at 230°C, and the electron multiplier voltage (EMV) was 1200 V.

HPLC Analysis of Thymoquinone

Thymoquinone content in the extract was quantified following the method by Soliman *et al.*[18] A 42.60 mg sample of the extract was dissolved in methanol, filtered using a 0.45 µm syringe filter, and analyzed by Shimadzu LC-20AD HPLC system. The injection volume was 10 µL. Quantification was based on a calibration curve constructed using thymoquinone standards, and the concentration was expressed as percentage weight per weight (% w/w). The chromatographic results were obtained from our previously published data.[19]

Cell Culture

The B16F10 mouse melanoma cell line (ATCC CRL-6475, USA) was supplied by the Primate Animal Research Centre, IPB University, Bogor, Indonesia. Cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin (10,000 U per100 mg mL), at 37 °C in an incubated with 5% CO2. Cells were trypsinized, sub-cultured, and treated with *N. sativa* extracts, IBMX and TQ with various concentrations of tested samples for 48 h. Control cells were cultured with complete DMEM media.

Cell Viability Assay

Cell viability was assessed using the MTT assay as described by Mosmann.[20] B16F10 cells were seeded into 96-well plates at 50% confluency and incubated for 18–20 h. Cells were treated with various concentrations of EE BCS (6.25–800 ppm), TQ (0.75–100 ppm), or IBMX (1.60–200 ppm). The TQ was used as a reference compound for comparison, while IBMX served as a positive control for melanogenesis. Untreated cells served as controls. After 48 h of incubation, 50 µg of MTT solution was added to each well, and the plates were incubated for an additional 4 h. The resulting formazan crystals were dissolved in 100 µL of 96% ethanol, and the supernatant was discarded. Absorbance was measured at 595 nm using a microplate reader (Bio-Rad, Japan). To determine the percentage of cell viability for each treatment, optical density data were analyzed. Cell viability (%) was calculated as follows: [21]

$$\% \text{ Cell Viability} = \frac{(\text{sample absorbance} - \text{blank absorbance})}{(\text{control absorbance} - \text{blank absorbance})} \times 100$$

Effect of ethanol extract of black cumin seeds (EE BCS) on tyrosinase activity and melanin content in B16F10 melanoma cells

Measurement of tyrosinase activity

Tyrosinase activity was measured using the L-DOPA oxidation method. B16F10 cells (5×10^5 cells/well) were seeded in 96-well plates and incubated for 24 h. Cells were treated with 100 µL of EE BCS (3.13, 6.25, and 12.50 ppm), TQ (0.25 ppm), or IBMX (6.25 ppm) for 48 h at 37°C in a 5% CO_2 atmosphere. After treatment, cells were washed with PBS and lysed with 0.1% Triton X-100 in Tris-HCl buffer (pH 7.5). The lysates were centrifuged, and the supernatants were incubated with 10 mM L-DOPA for 60 minutes at room temperature in the dark. Absorbance was measured at 450 nm using a microplate reader (Bio-Rad, Japan) to quantify dopaquinone formation. Tyrosinase activity was expressed as a percentage relative to untreated control. Untreated cells served as controls.

Measurement of cellular melanin content

Melanin content was determined in B16F10 cells seeded at 1×10^5 cells/well in 96-well plates. After 24 h, cells were treated with 100 µL of EE BCS (3.13, 6.25, and 12.50 ppm), TQ (0.25 ppm), or IBMX (6.25 ppm) for 48 h at 37°C in a 5% CO_2 environment. After incubation, cells were lysed in 1M NaOH and rinsed twice with PBS. The optical density of each lysate was measured at 450 nm using a microplate reader (Bio-Rad, Japan).[22] Melanin production as a proportion of the untreated control was used to express the results. The formula below was used to determine the percentage increase in melanin content in cultured B16F10 cells:

$$\% \text{ Melanin} = \frac{\text{Optical Density Test}}{\text{Optical Density Control}} \times 100\%$$

Ethical Considerations

As this study involved only *in-vitro* experiments using commercially available cell lines, no ethical clearance was required in accordance with institutional and national guidelines.

DATA ANALYSIS

All data were carried out triplicate and the results were presented as mean ± standard deviation. One-way ANOVA was used for multiple group comparisons, followed by the Mann–Whitney U test for post-

hoc analysis. P values less than 0.05 were considered to be statistically significant by utilizing the GraphPad Prism v10.4.0 (GraphPad Software Inc., USA).

RESULTS

GC-MS Analysis

The GC-MS analysis of the 96% ethanol extract of black cumin seeds (EE BCS) identified 20 phytochemical compounds. The major constituents were 9,12-Octadecadienoic acid - methyl ester (E,E) (45.88%), 9,12-octadecadienoic acid (Z,Z) (25.17%), and isopropyl linoleate (11.59%) (Table 1).

Thymoquinone Quantification by HPLC

HPLC analysis (Figure 1) confirmed the presence of TQ in EE BCS at 0.04% with a retention time of 3.35 min based on our previous studies.[19]

Effect of ethanol extract of black cumin seeds (EE BCS) on B16F10 cells viability

The viability of B16F10 melanoma cells was inversely proportional to the concentrations of EE BCS, TQ, and IBMX (Figure 2). The highest cell viability for EE BCS was observed at 6.25 ppm (97.61%), and the lowest was observed at 800 ppm (12.92%). A significant reduction in cell viability was observed at EE BCS 25 ppm ($p < 0.01$). For TQ, the highest viability was recorded at 0.75 ppm (59.06%) and the lowest at 100 ppm (2.69%), while IBMX exhibited the highest viability at 1.60 ppm (108.29%) and the lowest at 200 ppm (11.77%).

Cell viability can also be assessed by determining the IC_{50}, which reflects the potential toxicity of a compound and serves as the basis for establishing the maximum testing concentration. In this study, the maximum concentration required to achieve 50% cell viability was 50 ppm for EE BCS, 0.75 ppm for TQ, and 25 ppm for IBMX. Concentrations exceeding these values reduced the cell viability by more than 50%. EE BCS demonstrated over 90% cell viability at concentrations up to 12.50 ppm, with an IC_{50} value of 56.41 ppm. In comparison, the IC_{50} values of TQ and IBMX were 1.01 ppm and 26.41 ppm, respectively. The R^2 values for EE BCS, TQ, and IBMX were 0.96, 0.91, and 0.98, respectively, indicating strong linear correlations ($R^2 > 0.90$) between compound concentrations and cell viability.

Microscopic examination revealed a concentration-dependent alteration in cellular morphology following EE BCS treatment (Figure 3). In general, higher concentrations were associated with more pronounced morphological changes such as cytoplasmic condensation and evident shrinkage suggestive of cell death. These features were notably observed in cultures treated with EE BCS 100 ppm and above (Figures 3F – 3I). Minimal morphological signs of cytotoxicity were observed in cultures treated with EE BCS up to 50 ppm (Figure 3B – 3E).

Effect of ethanol extract of black cumin seeds (EE BCS) on tyrosinase activity in B16F10 cells.

Cells treated with EE BCS, TQ, and IBMX exhibited increased dopaquinone levels compared to the control. Significant differences ($p < 0.05$) were observed at concentrations of IBMX 6.25 ppm (139.60%), TQ 0.25 ppm (168.30%), and EE BCS 6.25 ppm (136.40%; $p < 0.05$). A highly significant increase ($p < 0.01$) was noted at an EE BCS of 12.50 ppm (228.10%) (Figure 4). No significant differences were detected between the increases in dopaquinone for EE BCS 6.25 ppm and 12.50 ppm compared to IBMX 6.25 ppm and TQ 0.25 ppm ($p > 0.05$), suggesting that the tyrosinase activity induced by EE BCS was comparable to that of IBMX and TQ. Conversely, EE BCS at 3.13 ppm resulted in a decrease in dopaquinone levels (95.05%), which was not significant, indicating no increase in tyrosinase activity at this concentration.

Effect of ethanol extract of black cumin seeds (EE BCS) on melanin content in B16F10 cells.

The melanin index was measured in B16F10 melanoma cells exposed to IBMX, TQ, and EE BCS. The melanin index represents the proportion of melanin relative to the total pigment content in cells compared to the control and is used to evaluate changes in melanin production due to specific treatments. An increased melanin index indicates higher melanin content within cells.[23,24]

A significant increase in the melanin index ($p < 0.05$) was observed in treated cells compared to the controls, with values of 191.30% for IBMX at 6.25 ppm, 221.40% for TQ at 0.25 ppm, and 123.80%, 226.00%, and 266.90% for EE BCS at 3.13, 6.25, and 12.50 ppm, respectively. The increase in EE BCS 12.50 ppm was highly significant ($p < 0.01$) (Figure 5). No significant differences were observed between the melanin index

Table 1: List of compounds in 96% ethanol extract of black cumin seeds.

No	Name of the Compound	Molecular Formula	Molecular Weight	Retention Time (min)	Area %	Height %
1	1,3-Octadiene	C_8H_{14}	112	5.959-6.084	1.28	1.61
2	9,12-Octadecadienal	$C_{18}H_{32}O_2$	270	35.429-35.488	1.49	1.61
3	9,12-Octadecadienoic acid - methyl ester (E,E)	$C_{19}H_{34}O_2$	294	36.817-36.918	45.88	50.00
4	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	270	40.078-40.178	25.17	30.32
5	Isopropyl Linoleate	$C_{21}H_{38}O_2$	314	42.022-42.084	11.59	15.24
6	Octamethylcyclotetrasiloxane	$C_8H_{24}O_4Si_4$	296	51.125-51.752	9.23	12.01
7	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	228	42.586-42.648	7.62	9.18
8	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	370	41.018-41.344	6.28	7.62
9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	36.855-36.955	5.18	6.54
10	Octadecanoic acid, 2-propenyl ester	$C_{21}H_{40}O_2$	324	39.037-39.087	3.22	4.10
11	Cyclooctane	C_8H_{16}	112	6.312	1.28	1.61
12	α-Phellandrene	$C_{10}H_{16}$	136	6.952	1.34	1.49
13	β-Phellandrene	$C_{10}H_{16}$	136	7.860	1.77	1.90
14	γ-Terpinene	$C_{10}H_{16}$	136	9.241	2.09	2.35
15	Terpinen-4-ol	$C_{10}H_{18}O$	154	11.921	2.22	2.50
16	Farnesane	$C_{15}H_{32}$	212	14.953	1.19	1.30
17	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	16.702	0.88	0.95
18	Methyl Myristate	$C_{15}H_{30}O_2$	242	18.071	0.75	0.84
19	Methyl Linoleate	$C_{19}H_{34}O_2$	294	23.716	1.34	1.49
20	Methyl Oleate	$C_{19}H_{36}O_2$	296	23.792	0.42	0.48

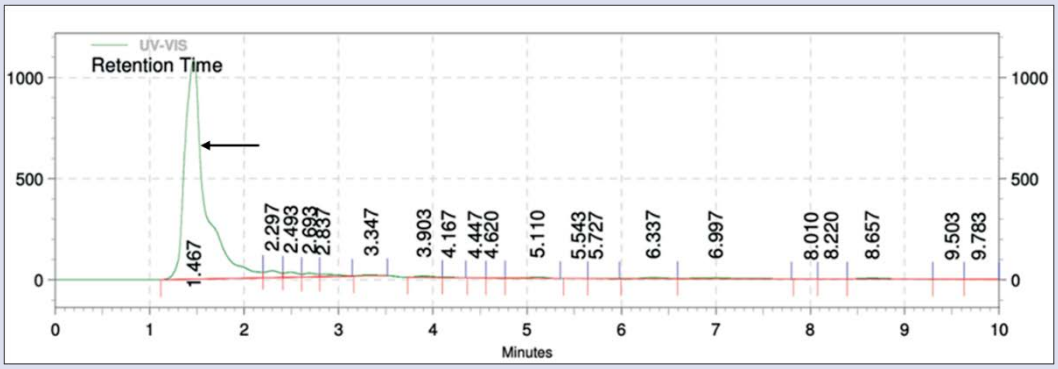

Figure 1: Chromatogram of 96% ethanol extract of black cumin seeds at 254 nm in HPLC analysis. The injection volume was 10 µL. ← Thymoquinone.[19]

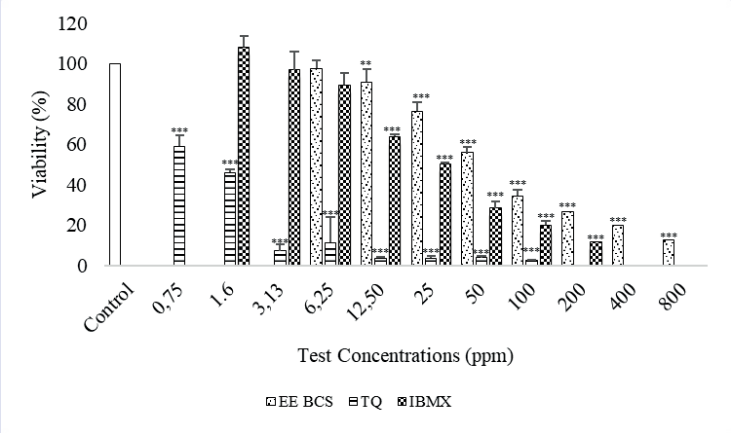

Figure 2: Percentage of B16F10 cell viability after treatment with 96% ethanol extract of black cumin seeds (EE BCS), thymoquinone (TQ), and isobutylmethylxanthine (IBMX) using MTT assay. Each bar represents the mean of three replicates with standard deviations. **p < 0.05, ***p < 0.01.

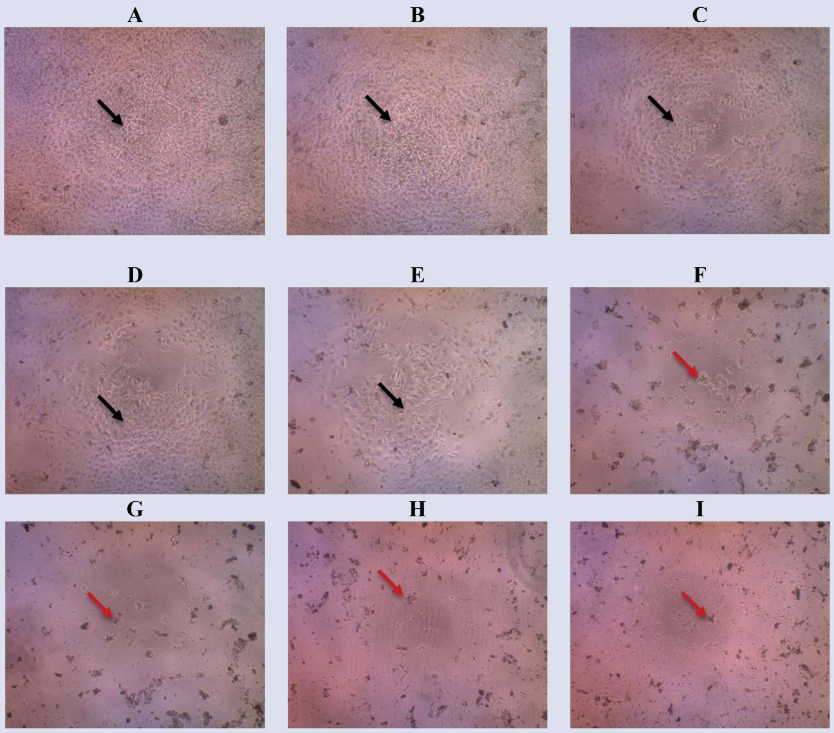

Figure 3: Morphology of B16F10 melanoma cells treated with EE BCS (32x magnification). Dose concentrations (A) control, (B) 6.25 ppm, (C) 12.5 ppm, (D) 25 ppm, (E) 50 ppm, (F) 100 ppm, (G) 200 ppm, (H) 400 ppm, and (I) 800 ppm. Live cells (→). Dead cells (→).

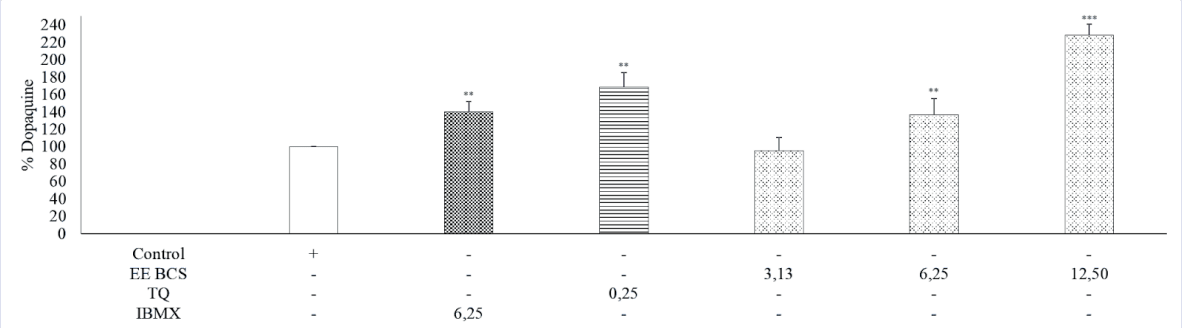

Figure 4: Percentage of dopaquinone in B16F10 cells treated with isobutylmethylxanthine (IBMX), thymoquinone (TQ), and 96% ethanol extract of black cumin seeds (EE BCS) using L-DOPA. Each bar represents the mean of three replicates with standard deviations shown as percentages of dopaquinone. **p < 0.05, ***p < 0.01.

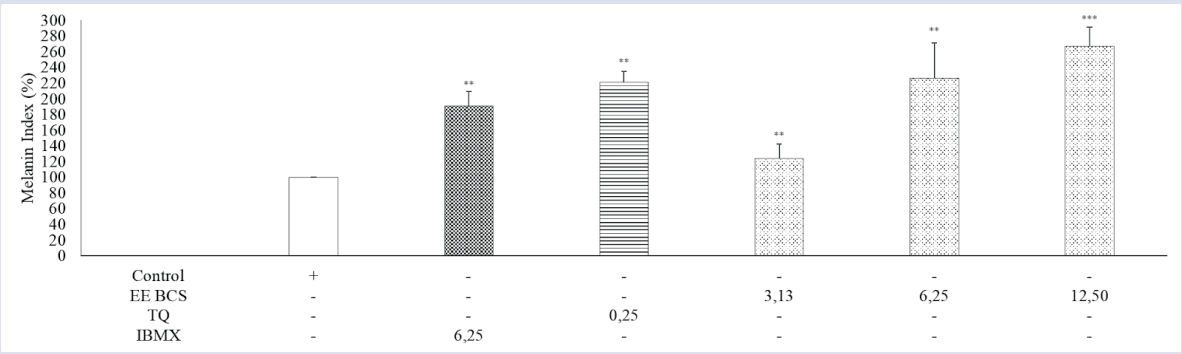

Figure 5: Percentage of melanin index of B16F10 cells with isobutylmethylxanthine (IBMX), thymoquinone (TQ), and 96% ethanol extract of black cumin seeds (EE BCS) treatments. Each bar represents the average of three replicates, with the standard deviation shown as % melanin index. **p<0.05, ***p < 0.01.

increases for EE BCS at 3.13, 6.25, and 12.50 ppm compared to IBMX at 6.25 ppm and TQ at 0.25 ppm (p > 0.05).

DISCUSSION

The biological effects of plant extracts are largely influenced by their phytochemical composition. In this study, GC-MS analysis was conducted to identify the major bioactive constituents potentially responsible for the observed pro-melanogenic activity. 9,12-Octadecadienoic acid - methyl ester (E,E), the predominant compound found in this study, is a methylated linoleic acid (omega-6 fatty acid) that has been reported to exhibit anti-inflammatory, anti-acne, antioxidant, antihistaminic, and anti-eczemic properties.[25] These findings are consistent with those of Hagr *et al.* in Sudan, who also identified this compound as the major component of the BCS extract (48.72%).[26] Its antioxidant and anti-inflammatory activities may be particularly relevant in vitiligo, where oxidative stress and inflammation are key contributors to melanocyte dysfunction and melanin loss.[2] In addition to linoleic acid derivatives, the extract also contains other bioactive, such as flavonoids and sterols,[19] which are known to modulate melanogenesis and exert antioxidant effects.[27,28] The combination of these bioactive constituents may act synergistically to protect melanocytes from oxidative damage and promote a microenvironment conducive to melanin production.

However, other studies from different regions reported varying chemical profiles. Hariutami *et al.* in Indonesia identified hexadecenoic acid ethyl ester (26.14%) as the primary compound in EE BCS [11], while Saleh *et al.* in Lebanon reported β-pinene (9.1%), o-cymene (8.7%), and thymoquinone (7.8%) as the three most abundant constituents in *N. sativa* oil.[29] In India, Ghadge *et al.* found that 9,12-octadecadienoic acid ethyl ester (44.14%), thymoquinone (20.54%), and hexadecanoic acid ethyl ester (11.30%) were the major components.[30] Variations in chemical composition may result from differences in geography, genotype, extraction methods, and solvents—highlighting the need for region-specific evaluations of therapeutic potential.[31]

Thymoquinone concentration in our extract (0.04%) is notably lower compared to imported BCS, such as those from India and the Middle East, which may contain up to 48% TQ.[7,31,32] This disparity may be attributed to environmental factors such as soil composition, climate, and genotype in *N. sativa* grown in Indonesia. The extraction technique and solvent type employed can also affect the concentration of thymoquinone in the final extract.[11] Previous studies have demonstrated that higher TQ levels in imported BCS contribute to stronger immunomodulatory effects associated with therapeutic benefits.[33] However, the lower TQ content in Indonesian BCS does not necessarily diminish its therapeutic potential, as other bioactive compounds may play a compensatory role in melanogenesis.[27] Whole-seed extracts may exert a more balanced and synergistic effect due to the presence of multiple bioactive constituents, which may compensate for lower TQ levels. These interactions could influence melanogenesis positively, which may offer advantages over isolated compounds, particularly in modulating complex biological processes such as melanogenesis[27,28], as shown in this study.

The MTT assay is a widely accepted colorimetric method for assessing cell viability and proliferation, and is considered a gold standard for cytotoxicity evaluation.[21,34] EE BCS exhibited relatively low cytotoxicity with an IC_{50} value of 56.41 ppm. The IC_{50} values of TQ and IBMX were 1.01 ppm and 26.41 ppm, respectively, indicating greater cytotoxicity of the isolated compound and positive control. The higher IC_{50} of EE BCS compared to TQ suggests that the extract is less cytotoxic and potentially safer for application in skin-related therapies. This is

particularly relevant for long-term topical use, where cellular toxicity must be minimal to preserve melanocyte function.

Morphological changes in cellular structure are considered indicative parameters of cytotoxic response following exposure to chemical or bioactive compounds.[35] Therefore, the cytotoxic effects of EE BCS on B16F10 melanoma cells were further evaluated based on observable changes in cell morphology. The B16F10 melanoma cell culture exhibited a heterogeneous morphology, characterized by a mixture of spindle-shaped and epithelial-like cells, as seen in control (Figure 3A). Microscopic examination revealed a concentration-dependent alteration in cellular morphology following EE BCS treatment. In general, higher concentrations were associated with more pronounced morphological changes, suggestive of cytotoxic effects. Such cytotoxic responses in melanoma cells are characterized by cellular shrinkage, detachment from the culture surface, reduction in cell and nuclear volume, an increase in floating or non-adherent cells, cytoplasmic condensation, and evident shrinkage suggestive of cell death.[36] These features were notably observed in cultures treated with EE BCS 100 ppm and above (Figures 3F – 3I). In contrast, minimal morphological signs of cytotoxicity were observed in cultures treated with EE BCS up to 50 ppm (Figure 3B – 3E), suggesting minimal cytotoxicity. This observation is consistent with MTT assay results, which showed the maximum concentration required to achieve 50% cell viability was 50 ppm. Akinwumi *et al.* also reported normal liver and kidney histology after 50 ppm EE BCS, supporting its low toxicity profile.[37]

Melanin synthesis begins with the conversion of L-tyrosine to L-DOPA by tyrosinase, followed by the conversion of L-DOPA to dopaquinone.[38] Dopaquinone levels are commonly measured to evaluate tyrosinase activity following the administration of L-DOPA to enzymes exposed to the test compounds.[22] In this study, EE BCS enhanced tyrosinase activity in a concentration-dependent manner, suggesting its potential as a melanogenesis-stimulating agent. The findings align with study by Ben *et al.* in China, who reported that EE BCS at concentrations of 1, 10, and 20 ppm significantly increased tyrosinase activity in B16F10 cells, with increases observed at 1 ($p < 0.01$), 10 ($p < 0.05$), and 20 ppm ($p < 0.001$).[39] Variations in the effectiveness of EE BCS in enhancing tyrosinase activity may be attributed to differences in the types and concentrations of secondary metabolites, influenced by geographical and climatic factors.[31] Notably, while our study observed an increase in tyrosinase activity with EE BCS treatment, other studies have reported the inhibitory effects of certain *N. sativa* extracts on melanogenesis. Li *et al.* found that Thymocid®, a standardized black cumin seed extract, decreased melanin content and reduced cellular tyrosinase activity in B16F10 cells.[40] This discrepancy may be attributed to the significant difference in TQ content. While Thymocid® contains 5.12% TQ, our EE BCS extract had only 0.04% TQ. Additionally, variations in extraction methods, solvents, cultivation conditions, and geographical origin may influence the composition of other bioactive compounds, contributing to differential effects on tyrosinase activity.

The ability of EE BCS to enhance melanin production suggests a stimulatory effect on the melanogenesis pathway. The findings are consistent with study by Ben *et al.*, who reported a significant increase in melanin production in B16F10 cells exposed to 1 and 10 ppm EE BCS for 48 h ($p < 0.001$).[39] EE BCS enhanced melanin production to levels comparable to IBMX and TQ, confirming its melanogenesis-promoting effect. Studies have shown that TQ exhibits biphasic, concentration-dependent effects on melanogenesis. Zaidi *et al.* reported that low-concentration TQ (0,16 – 0,82 ppm) significantly enhanced tyrosinase activity and melanin production in B16F10 cells, while Jeong *et al.* found that higher concentrations of TQ (1,64 – 3,28 ppm) inhibited tyrosinase activity and melanin synthesis through suppression of the Wnt/β-catenin pathway, indicating a concentration-dependent shift toward anti-melanogenic effects. This hormetic response suggests that TQ may be pro-melanogenic at lower concentration and inhibitory at higher levels.[27,41]

Given the extract's relatively low TQ content (0.04%) and predominant levels of linoleic acid derivatives, 9,12-octadecadienoic acid methyl ester (E,E) (45.88%), Indonesian *Nigella sativa* may offer a favorable profile for vitiligo therapy, combining pro-melanogenic activity with anti-inflammatory and antioxidant protection. Owing to its local availability and traditional use in Indonesia, EE BCS also offers socio-economic advantages. This further highlights the importance of evaluating region-specific whole-plant extracts in contrast to isolated compounds. The variability in tyrosinase activity and melanin synthesis across *Nigella sativa* extracts highlights the importance of standardizing TQ levels and other key bioactive compounds to ensure consistency and reproducibility of the desired biological effects, particularly the stimulation of tyrosinase activity and melanogenesis.

CONCLUSION

The 96% ethanol extract of *Nigella sativa* seeds significantly increased tyrosinase activity and melanin production in B16F10 cells at non-cytotoxic concentrations. This study also highlights the potential of *Nigella sativa* cultivated in Indonesia as a region-specific phytotherapeutic agent, whose unique phytochemical composition—characterized by low TQ and abundant anti-inflammatory constituents—may offer tailored dermatological benefits. Given these attributes, Indonesian *Nigella sativa* represents a compelling candidate for further development into a dermatological product targeting vitiligo. To support its translational potential, subsequent *in-vivo* and clinical research is needed to establish both its efficacy and safety.

ACKNOWLEDGEMENTS

The authors would like to thank all the promoters and all those who have helped in the implementation of this research.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

REFERENCES

1. Jacoeb TNI. 'Vitiligo'. In Menaldi S.L.S.W. (ed), Bramono K. (ed) and Indriatmi W. (ed) Ilmu penyakit kulit dan kelamin. 7th ed. Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia, p. 352–358. 2021. *In Indonesian*.
2. Davis CC, Choisy P. Medicinal plants meet modern biodiversity science. Curr Biol [Internet]. 2024;34(4):R158–73. Available from: https://doi.org/10.1016/j.cub.2023.12.038
3. Dabeer S, Rather MA, Rasool S, Rehman MU, Alshahrani S, Jahan S, *et al.* History and traditional uses of black seeds (*Nigella sativa*). In: Black Seeds (*Nigella sativa*). Amsterdam: Elsevier; 2022. p. 1–28.
4. Mardisiwi RS, Kurniawati A, Sulistyono E, Fardidah DDN. Pertumbuhan dan Produksi Jintan Hitam pada Beberapa Komposisi Media Tanam dan Interval Penyiraman. J Agron Indones. 2018;46(1):89. *In Indonesian.*
5. Dhaheri Y Al, Wali AF, Akbar I, Rasool S, Razmpoor M, Jabnoun S, *et al. Nigella sativa*, a cure for every disease: Phytochemistry, biological activities, and clinical trials. Pharmacol Ther Appl. 2022;63–90.
6. Javed S, Sultan MH, Ahsan W, Khan A. Dermatological effects of *Nigella sativa*: A cosmetic and therapeutic approach. In: Black Seeds (*Nigella sativa*). Amsterdam: Elsevier; 2022. p. 119–48.
7. Ravi Y, Periyanadar IV, Saxena SN, Muthurajan R, Sundararajan V, Pridiuldi SV, *et al.* Identification, validation and quantification of thymoquinone in conjunction with assessment of bioactive possessions and GC-MS profiling of pharmaceutically valuable crop Nigella (*Nigella sativa* L.) varieties. PeerJ. 2024;12.

8. Majdalawieh AF, Fayyad MW. Immunomodulatory and anti-inflammatory action of *Nigella sativa* and thymoquinone: A comprehensive review. Int Immunopharmacol [Internet]. 2015;28(1):295–304. Available from: http://dx.doi.org/10.1016/j.intimp.2015.06.023
9. Khan J, Ali A, Balyan P, Bhat EA. Role of *Nigella sativa* as immunomodulator. In: Black Seeds (*Nigella sativa*). Amsterdam: Elsevier; 2022. p. 337–54.
10. Mehraj T, Elkanayati RM, Farooq I, Mir TM. A review of *Nigella sativa* and its active principles as anticancer agents. In: Black Seeds (*Nigella sativa*). Amsterdam: Elsevier; 2022. p. 91–118.
11. Hariutami D, Darmawati S, Permana A, Zuraida Z. The Effect of Different Solvents on the Content of Black Cumin Seed Extract (*Nigella sativa*). J Kefarmasian Indones. 2024;14(February):84–97.
12. Elzahrani M, Aleid A, Alhwimani A, Altamamy S, Almalki R, Alkushi A, *et al.* Perceptions, knowledge, and attitudes toward complementary and alternative medicine among saudi patients with vitiligo. Acta Dermatovenerologica Alpina, Pannonica Adriat. 2020;25(4):165–8.
13. Ghorbanibirgani A, Khalili A, Rokhafrooz D. Comparing *Nigella sativa* oil and fish oil in treatment of vitiligo. Iran Red Crescent Med J. 2014;16(6).
14. Sarac G, Kapicioglu Y, Sener S, Mantar I, Yologlu S, Dundar C, *et al.* Effectiveness of topical *Nigella sativa* for vitiligo treatment. Dermatol Ther. 2019;32(4):1–4.
15. Setiana WS. Perbandingan farmakognisi, profil kromatografi lapis tipis dan kadar minyak atsiri biji jinten hitam lokal dan impor. Thesis, Universitas Pancasila, Indonesia, 2010. *In Indonesian.*
16. Kim T, Hyun CG. Imperatorin Positively Regulates Melanogenesis through Signaling Pathways Involving PKA/CREB, ERK, AKT, and GSK3 β / β -Catenin. Molecules [Internet]. 2022;27(19):6512. Available from: doi: 10.3390/molecules27196512
17. Laksmiani NPL, Sanjaya IKN, Leliqia NPE. The activity of avocado (*Persea americana* Mill.) seed extract containing catechin as a skin lightening agent. J Pharm Pharmacogn Res. 2020;8(5):449–56.
18. Soliman RM, Abdel Salam RA, Eid BG, Khayyat A, Neamatallah T, Mesbah MK, *et al.* Stability study of thymoquinone, carvacrol and thymol using HPLC-UV and LC-ESI-MS. Acta Pharm. 2020;70(3):325–42.
19. Pratiwi D, Mariya S, Rayendra R, Setiyono A. Evaluating the Cytotoxic Effects of Ethanol and n-Hexane Extracts from Black Cumin Seeds (*Nigella sativa*) on B16F10 Mouse Melanoma Cells : A Preliminary Investigation into Vitiligo Treatment. HAYATI J Biosci. 2025;32(1):203–11.
20. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. J Immunological Methods. 1983;65:55–63.
21. Kamiloglu S, Sari G, Ozdal T, Capanoglu E. Guidelines for cell viability assays. Food Front. 2020;1(3):332–49.
22. Widyastuti W, Putra DP, Yenny SW, Elliyanti A. In vitro study: Catechins as depigmenting agents inhibit melanogenesis on B16F0 cells. J Appl Pharm Sci. 2023;13(6):237–45.
23. Del Bino S, Duval C, Bernerd F. Clinical and biological characterization of skin pigmentation diversity and its consequences on UV impact. Int J Mol Sci. 2018;19(9).
24. Kanellis VG. A review of melanin sensor devices. Biophys Rev. 2019;11(6):843–9.
25. Akeem A, Ekunseitan DA, Oguntoye M. Phytochemicals Analysis and GC-MS Determination of Ethanolic Extracts of Azadirachta indica and Mangifera indica Stem Bark and their Biological Potentials. Pacific J Sci Technol [Internet]. 2020;21(1):219–29. Available from: http://www.akamaiuniversity.us/PJST.htm
26. Hagr TE, Ali KS, Satti AAE, Omer SA. GC-MS analysis, phytochemical, and antimicrobial activity of Sudanese *Nigella sativa* (L) oil. Eur J Biomed Pharm Sci. 2018;5(4):1–7.
27. Jeong H, Yu SM, Kim SJ. Inhibitory effects on melanogenesis by thymoquinone are mediated through the β -catenin pathway in B16F10 mouse melanoma cells. Int J Oncol. 2020;56(1):379–89.
28. Shin S, Ko J, Kim M, Song N, Park K. Morin induces melanogenesis via activation of mapk signaling pathways in b16f10 mouse melanoma cells. Molecules. 2021;26(8).
29. Saleh FA, El-Darra N, Raafat K, El Ghazzawi I. Phytochemical analysis of *Nigella sativa* L. Utilizing GC-MS exploring its antimicrobial effects against multidrug-resistant bacteria. Pharmacogn J. 2018;10(1):99–105.
30. Ghadge RS, Panchbhai CG, Dhoot VM, Bhojne GR, Somkuwar AP. Gas chromatography-mass spectrometry analysis of *Nigella sativa* black cumin seeds. Int J Vet Sci Anim Husb. 2024;9(10585):302–5.
31. Gharby S, Harhar H, Guillaume D, Roudani A, Boulbaroud S, Ibrahimi M, *et al.* Chemical investigation of *Nigella sativa* L. seed oil produced in Morocco. J Saudi Soc Agric Sci [Internet]. 2015;14(2):172–7. Available from: http://dx.doi.org/10.1016/j.cdnut.2023.100471
32. Goyal SN, Prajapati CP, Gore PR, Patil CR, Mahajan UB, Sharma C, *et al.* Therapeutic potential and pharmaceutical development of thymoquinone: A multitargeted molecule of natural origin. Front Pharmacol. 2017;8(SEP):1–19.
33. Koshak AE, Yousif NM, Fiebich BL, Koshak EA, Heinrich M. Comparative immunomodulatory activity of *Nigella sativa* L. preparations on proinflammatory mediators: A focus on asthma. Front Pharmacol. 2018;9(OCT):1–11.
34. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT Assay : Utility , Limitations , Pitfalls , and Interpretation in Bulk and Single-Cell Analysis. Int J Mol Sci [Internet]. 2021;22(12827). Available from: https://doi.org/10.3390/ijms222312827
35. Aisyah AN, Nur S, Lukitaningsih E, Rumiyati R, Burhan A, Adjara SM, *et al.* Efek Sitotoksik Ekstrak dan Fraksi Umbi Paku Atai Merah (Angiopteris ferox Copel) Terhadap Sel Kanker Payudara T47D. J Farm Galen (Galenika J Pharmacy). 2020;6(2):319–27. *In Indonesian.*
36. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. Cold Spring Harb Protoc. 2018;2018(6):469–71.
37. Akinwumi KA, Jubril AJ, Olaniyan OO, Umar YY. Ethanol extract of *Nigella sativa* has antioxidant and ameliorative effect against nickel chloride-induced hepato-renal injury in rats. Clin Phytoscience. 2020;6(1).
38. Wang F, Ma W, Fan D, Hu J, An X, Wang Z. The biochemistry of melanogenesis: an insight into the function and mechanism of melanogenesis-related proteins. Front Mol Biosci. 2024;11(August):1–15.
39. Ben N, Xiaohong A, Yongmei C, Ting H, Xiao Z, Xiuqi Z, *et al. Nigella sativa* L. seed extract alleviates oxidative stress-induced cellular senescence and dysfunction in melanocytes. Chin J Nat Med. 2024;22(x):1–14.
40. Li H, Dasilva NA, Liu W, Xu J, Dombi GW, Dain JA, *et al.* Thymocid®, a standardized black cumin (*Nigella sativa*) seed extract, modulates collagen cross-linking, collagenase and elastase activities, and melanogenesis in murine B16F10 melanoma cells. Nutrients. 2020;12(7):1–16.
41. Zaidi KU, Khan FN, Ali SA, Khan KP. Insight into Mechanistic Action of Thymoquinone Induced Melanogenesis in Cultured Melanocytes. Protein Pept Lett. 2019;26(12):910–8.

Cite this article: Pratiwi D, Mariya S, Rayendra R, Setiyono A. Phytochemical Analysis and Pro-Melanogenic Activity of *Nigella sativa* Extract in B16F10 Cells: A Natural Candidate for Vitiligo Treatment. Pharmacogn J. 2025;17(3): 307-313.