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Assessment of Antimicrobial and Antioxidant Efficacy of SCFE-CO_2 Extracted *Nigella sativa* L. Seed Oil

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ABSTRACT

Background: This study investigates the antimicrobial and antioxidant potential of thymoquinone (TQ) rich *Nigella sativa* L. seed oil (NSO) extracted using supercritical carbon dioxide extraction (SCFE-CO_2)

Methods: *N. sativa* seeds were ground, sieved (Mesh No. 24) and subjected to SCFE using CO_2 at extraction conditions 40°C, 20 MPa and 0.71 × 10^{-3} m particle size. The extracted oil was characterized for thymoquinone (TQ) content using GC-MS/MS and microstructural differences between raw and defatted seeds post-extraction using Scanning Electron Microscopy (SEM) analysis at 1 KX magnification. Antibacterial efficacy was assessed against five pathogenic strains (*Listeria monocytogenes* ATCC 13932*, Staphylococcus aureus* ATCC 6538*, Escherichia coli* ATCC 25922*, Vibrio parahaemolyticus* ATCC 17802 and *Pseudomonas aeruginosa* ATCC 9027) using the disc diffusion method, while antioxidant activity was evaluated using the DPPH radical scavenging assay.

Result: The extracted oil yielded 18.8% (w/w) with a TQ content of 3.62%. The microstructural matrix of defatted seeds confirmed the effective extraction of oil and its bioactives, showing a lesser oil fraction residue. Zones of inhibition (mm) at 100 µL and 200 µL for *Listeria monocytogenes* were 13±0.09 and 15.3±0.05*;* for *Staphylococcus aureus,* 10±0.20 and 13.3±0.06; for *Escherichia coli,* 12±0.15 and 16.6±0.12; for *Vibrio parahaemolyticus,* 10±0.32 and 15±0.08; for *Pseudomonas aeruginosa,* 14±0.10 and 18.2±0.07. NSO exhibited strong antioxidant activity, with 96.76% radical scavenging activity (RSA).

Key words: Antimicrobial, Antioxidant, GC-MS/MS, *Nigella sativa* L., Seed oil, SEM, SCFE-CO_2, Thymoquinone.

INTRODUCTION

Therapeutic and aromatic plants have always been an integral part of traditional healing systems from ancient times. These plants are valuable sources of pharmaceutically active compounds, including alkaloids, flavonoids, carotenoids, tannins, saponins and essential oils. (Elujoba *et al*., 2005; Sing *et al*., 2014; Pandey *et al*., 2025). Despite having a therapeutic potential, scientific research remained limited, with only one-fifth of plants examined for biological activity and an even smaller fraction analyzed for their phytochemical composition (Salhi *et al*., 2011; Surya *et al*., 2024; Rabee *et al*., 2025).

Among all plants, *Nigella sativa* L. has received inclusive research attention, also referred to as black cumin, black caraway, or kalonji (Öz and Mustafa, 2018). Widely distributed across Western Asia, the Mediterranean and part of Eastern Europe, it has long been traditionally valued both as a culinary spice and as a remedy for numerous diseases (Burdock, 2022; Zouirech *et al*., 2022; Rabiej-Kozioł *et al*., 2024; Chatterjee *et al*., 2025). Its seeds and oil are especially renowned for their antioxidant, antimicrobial and anti-inflammatory properties (Atta and Mohamed Bassim, 2003; Singh *et al*., 2014; Thakur *et al*., 2021). The oil, supplemented with thymoquinone (TQ), flavonoids and essential fatty acids, has been studied for potential applications in pharmaceuticals, nutraceuticals, cosmeceuticals and functional foods (Ambati and Ramadan, 2021; Kour and Gani, 2020; Haffez *et al*., 2024).

In this study, the extraction of thymoquinone-rich *N. sativa* seed oil is done using the SCFE-CO_2 extraction technique at conditions of 20 MPa, 40°C and 0.71 × 10^{-3} m particle size to investigate the antimicrobial and antioxidant activities of *N. sativa* seed oil (NSO). (Solati *et al*., 2012; Salea *et al*., 2013; Gawron *et al*., 2021; Farhan *et al*., 2021). Salea *et al*. (2013) optimized the SCFE-CO_2 extraction of *Nigella sativa* seeds using the Taguchi method and full factorial design (FFD). The tested condition included 150-250 bar pressure, 40-60°C temperature and 10-20 g/min CO_2 flow rate. FFD produced a comparatively

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higher yield of 12% at 250 bar, 60°C and 20 g/min, while the Taguchi method achieved 11.9% yield at 250 bar, 50°C and 10 g/min. EP2209879B1 patent describes the extractions of fixed oil and thymoquinone rich fractions (TQRF) from *Nigella Sativa* seeds by supercritical carbon dioxide extraction (SCFE), where the extraction parameters were ranged between 31-80°C temperature and 300-600 bars pressure and resulted in an oil yield of 36.87% (w/w) at 600bars/80°C and TQ content of 0.59 g/100 g. (Ismail *et al*., 2008). US Pat. No. 8535740B2 entitled "Composition from *Nigella Sativa*" discloses novel supercritical fluid extracts of *Nigella Sativa* seeds containing about 0.01% to 40% (w/w) TQ and its antioxidant, thermogenic, anti-inflammatory and other bioactivities. However, the extraction of seeds resulted in low TQ content of 2.95% at 100 g and 39.3% at 100 kg. (Babish *et al*., 2013; El-Sayed *et al*., 2019).

Jiang *et al*. (2024) demonstrated the antiviral activity of black cumin seed oil against BCoV and HCoV OC43, achieving up to 3.0 logs of inhibition at 37°C within 60 minutes. HCoV OC43 was suggested as a better surrogate for SARS-CoV-2 (Jiang, Huang and Wang, 2024). *N. sativa* seed extract was found to improve pancreatic, liver and kidney function, regulate hyperglycemia and suppress genes related to pancreatic damage in streptozotocin (STZ) induced diabetic models, suggesting its therapeutic potential for diabetes management (Razni *et al*., 2019; Kim *et al*., 2025). TQ alle*via*ted cardiac hypertrophy in mouse and cell models by enhancing PPAR-γ/14-3-3γ signaling axis, reducing fibrosis and oxidative stress and improving cardiac function (Qiu *et al*., 2025). TQ is a potential radioprotector and anticancer agent (Fomina *et al*., 2024). Considering the importance of TQ in *N. sativa* seed oil, the objective of the present study is to overcome the limitation of prior art by providing supercritical CO_2-extracted TQ-rich *N. sativa* seed oil and also investigating its antimicrobial and antioxidant potential through *in vitro* evaluations (Chalghoumi *et al*., 2020; Naik *et al*., 2021). The quantification of TQ was determined using GC-MS/MS and the microstructural study was performed using SEM analysis to characterize the raw *N. sativa* seed and defatted *N. sativa* seed post SCFE-CO_2 extraction of seed oil. The research work advocates the importance of SCFE-CO_2 extraction as an efficient technology to obtain high antioxidant potential and improved antimicrobial efficacy of black cumin seed oil for therapeutic applications (Abbas *et al*., 2024; Soussa *et al*., 2024).

MATERIALS AND METHODS

The experiment was conducted during the 2024-2025 session in the laboratory of the Department of Food Technology at Haldia Institute of Technology, Haldia, West Bengal, India. High-quality *N. sativa* seed was procured from a Spice Board-approved vendor, SEED Agri Tech Pvt. Ltd., Kerala, India. *N. sativa* seed oil (NSO) was extracted using the supercritical carbon dioxide extraction (SCFE-CO_2) method, where the CO_2 (>99.9%) was purchased from Bharat Oxytech Pvt. Ltd., Haldia, West Bengal, India. *N. sativa* seeds were finely ground for 50-60 seconds to achieve a particle size of 0.71 × 10-3 m and sieved using a Mesh No. 24 to ensure uniform particle size distribution (Duru *et al*., 2021). After sieving, 1000 g of finely ground *N. sativa* seed powder is loaded into the extraction vessel. CO_2 is converted into a supercritical state (31.1°C, 7.39 MPa) (Zarrinpashne and Kendi, 2018). NSO was extracted with an optimized extraction condition at 40°C temperature, 20 MPa pressure and 0.71 × 10^{-3} m particle size, keeping the CO_2 flow rate constant at 12 g/min. After extraction, NSO is collected from the bottom valves of the separator vessels by separating the supercritical CO_2. TQ content in SCFE-CO_2 extracted NSO was characterized using a GC-MS/MS (Single Ion Monitoring/SIM Method) (Make and Model: AGILENT- 7000 MS and 7890 A-GC) and Column DB 5MS of dimensions 30 m × 0.25 µm × 0.25 µm. Microstructural characterization of *N. sativa* seed and defatted *N. sativa* seed after SCFE-CO_2 extraction at 1 KX magnification was done using Carl Zeiss GeminiSEM 360 - Germany (Türedi *et al*., 2022; Ravindran *et al*., 2025).

The antibacterial activity of TQ-rich *N. sativa* seed oil was evaluated against five reference pathogenic bacterial strains (*Listeria monocytogenes* ATCC 13932, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Vibrio parahaemolyticus* ATCC 17802 and *Pseudomonas aeruginosa* ATCC 9027) using the disc diffusion method. These strains were procured from HiMedia Laboratories Pvt. Ltd., India and subcultured onto fresh nutrient agar (NA). A bacterial suspension of each strain was prepared by transferring 5 colonies into 3 mL of distilled water (DW. 100 µL and 200 µL of each suspension were evenly spread on NA plates separately after 5 minutes. Sterile 5 mm paper discs (Whatman No. 15) were placed on the inoculated plates. Inhibitory activity of the TQ-rich *N. sativa* seed oil sample was tested by pipetting 10 µL of it onto the disc. All plates were incubated at 37±1°C for 18-24 hours. A digital vernier calliper was used to measure the zone of inhibition (Vakte and Nehete, 2025).

The antioxidant activity of NSO was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity (RSA) based spectrophotometric method. Reduction of DPPH in methanol by antioxidant compounds acting as hydrogen donors results in the formation of the non-radical DPPH form. A sample solution was prepared by dissolving NSO in dimethylsulfoxide. The sample is then mixed with DPPH solution and incubated in the dark for 30 minutes. Optical density (OD) was recorded at 715 n (Rahman *et al*., 2015). This method involves observing the color change of DPPH when reacted with an antioxidant, wherein the deep purple color of the DPPH solution fades to yellow upon reduction. The DPPH assay method was reported as %RSA using the equation (1) (Kumar *et al*., 2019 and Xiao *et al*., 2020; Zouirech *et al*., 2024):

$$\% \text{ RSA} = \frac{\text{Control OD - Oil sample OD}}{\text{Control OD}} \times 100$$

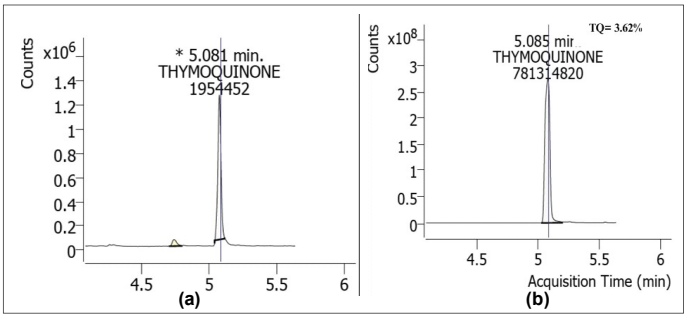

Fig 1: GC-MS/MS (SIM mode) analysis result of thymoquinone content in (a) standard thymoquinone and (b) thymoquinone in SCFE-CO_2 extracted NSO with extraction conditions at 20 MPa, 40°C and 0.71 × 10^{-3} m particle size.

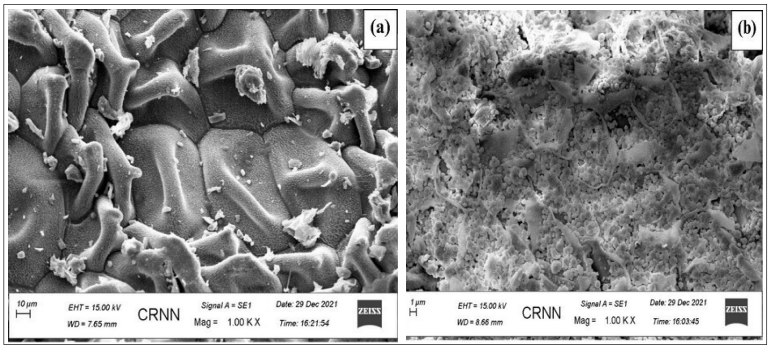

Fig 2: Microstructural study using Scanning Electron Microscopy (SEM) at 1 KX magnification of (a) *N. sativa* seed and (b) post SCFE-CO_2 extracted defatted *N. sativa* seed.

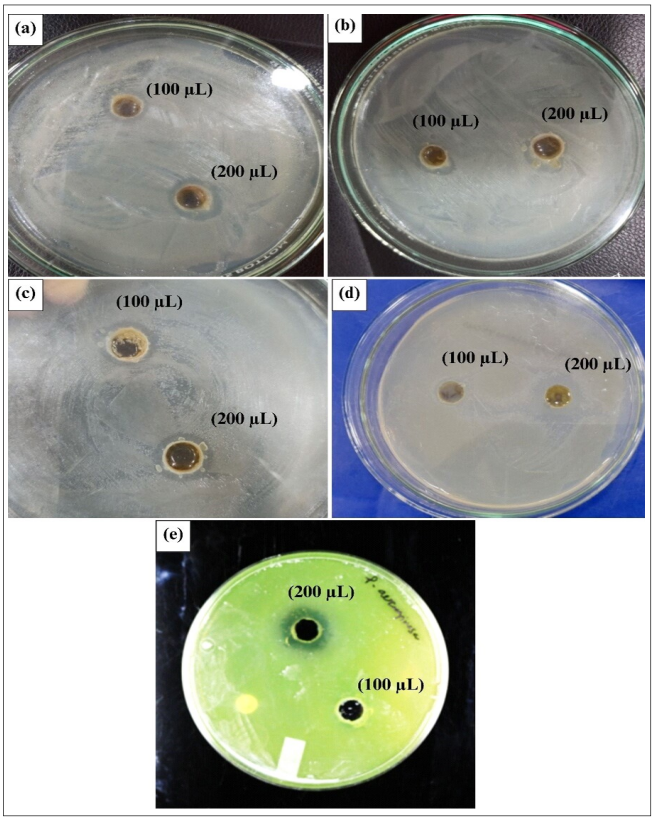

Fig 3: Anti-bacterial activity of NSO containing thymoquinone against (a) *Listeria monocytogenes,* (b) *Staphylococcus aureus,* (c) *Escherichia coli,* (d) *Vibrio parahaemolyticus and* (e) *Pseudomonas aeruginosa* in respect to 100 µL and 200 µL dosages.

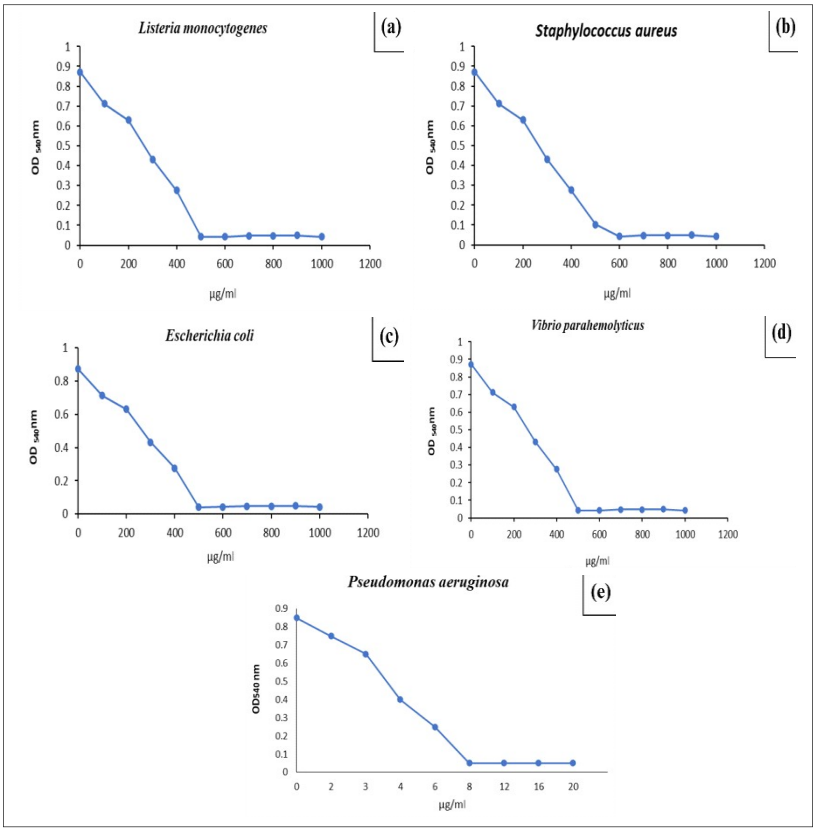

Fig 4: MIC at optical density 540 nm a) *Listeria monocytogenes,* (b) *Staphylococcus aureus,* (c) *Escherichia coli,* (d) *Vibrio parahaemolyticus and* (e) *Pseudomonas aeruginosa.*

Table 1: Anti-bacterial activities of TQ-rich *N. sativa* seed oil against the respective pathogenic bacterial strains.

Pathogens	Zone of inhibition (mm) with respect to different dosages	
	100 ul	200 ul
Listeria monocytogenes	13±0.09	15.3±0.05
Staphylococcus aureus	10±0.20	13.3±0.06
Escherichia coli	12±0.15	16.6±0.12
Vibrio parahaemolyticus	10±0.32	15±0.08
Pseudomonas aeruginosa	14±0.10	18.2±0.07

RESULTS AND DISCUSSION

Quantitative determination of TQ using the GC-MS/MS method

GC-MS/MS Scan data, as represented in Fig 1, shows that at a retention time (RT) of 5.085 min, 3.62% TQ is observed in the SCFE-CO_2 extracted NSO against standard TQ.

Microstructural characterization of *N. sativa* seed and defatted *N. sativa* seed post SCFE-CO_2 extraction using Scanning Electron Microscopy (SEM)

Micrographs represented in Fig 2 (a) show a well-defined compact cell structure of *N. sativa* seed at 1KX magnification, whereas Fig 2 (b) shows the disintegrated structure of cell walls with irregular texture of post SCFE-CO_2 extracted defatted *N. sativa* seed, confirming the effective extraction of oil and its bioactives and showing lesser oil fraction residue (Yildrim *et al*., 2024).

Antibacterial assay and minimum inhibitory concentration (MIC)

The antibacterial activity of TQ-rich *N. sativa* seed oil against *L. monocytogenes, S. aureus, E. coli, V. parahaemolyticus* and *P. aeruginosa* is represented in Fig 3. Zones of inhibition at 100 µL and 200 µL dosages for *Listeria monocytogenes* were 13±0.09 mm and 15.3±0.05 mm*;* for *Staphylococcus aureus,* 10 ± 0.20 mm and 13.3±0.06 mm; for *Escherichia coli,* 12±0.15 mm and 16.6±0.12 mm; for *Vibrio parahaemolyticus,* 10±0.32 mm and 15±0.08 mm; for *Pseudomonas aeruginosa,* 14±0.10 mm and 18.2±0.07 mm (Table 1). MIC was determined by reading the optical density (OD) at 540 nm Fig 4. MIC value for *L. monocytogenes* is 400 µg/mL, *S. aureus* 600 µg/mL, *E. coli* 500 µg/mL, *V. parahaemolyticus* 500 µg/mL and *P. aeruginosa* 8 µg/mL, respectively. On the contrary, we have concluded that the black cumin extracted oil has minimal to moderate inhibitory antibacterial activity against the selected pathogens (Bhaisare *et al*., 2016; Abo-Neima *et al*., 2023).

RSA activity of SCFE-CO_2 extracted N. sativa seed oil

NSO resulted in a significantly lower OD value of 0.051 compared to the control (1.576), indicating profound

scavenging of DPPH radicals. This corresponds to 96.76% RSA of the oil sample when calculated using equation 1, confirming the strong antioxidant potential of NSO (Alrashidi *et al*., 2022).

CONCLUSION

The present study investigated the antimicrobial and antioxidant efficacies of NSO extracted using SCFE-CO_2 technology. SCFE-CO_2 provides enhanced purity, selectivity and safety in extracting bioactive compounds, setting a benchmark for modern, sustainable green extraction Technologies in various industries have advanced significantly. Under optimized extraction conditions of 40°C, 20 MPa and a particle size of 0.71 × 10^(-3) m, the extracted oil yielded 18.8% (w/w) with a TQ content of 3.62%. It was subsequently characterized using GC-MS/MS. This research highlights the TQ-rich NSO extracted with SCFE-CO_2 technology as a promising commercial green technology with major applications in food, pharmaceutical, cosmeceutical and nutraceutical sectors due to its notable antimicrobial and antioxidant activities.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article, funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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