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Fatty Acid Profiling and Thymoquinone Quantification of Cold-Pressed Oils from Turkish- and Syrian-Originated *Nigella sativa* Seeds

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Abstract: *Nigella sativa* L. (black cumin) is an annual herb of the Ranunculaceae family, traditionally used in Middle Eastern and Asian medicine for the management of various health conditions. Its seeds and oils are rich in bioactive compounds, including alkaloids, essential fatty acids, nigellone, saponins, and thymoquinone (TQ), which contribute to diverse pharmacological activities such as anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant and hepatoprotective effects. In the present study, oils obtained from Turkish and Syrian *N. sativa* seeds through cold pressing were investigated. Phytochemical composition was characterized using Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC), with particular focus on fatty acid profiles and TQ content. Additionally, tyrosinase inhibitory activities of the fixed oils and TQ were evaluated. It was noted that variations in the composition of seed samples originating from different countries influence the phytochemical features of the plant materials. Accordingly, maintaining genetic diversity and implementing appropriate cultivation strategies are essential for the sustainability of agricultural production.

Keywords: cold-pressed oil; fatty acids; *Nigella sativa*; phytochemical analysis; thymoquinone; tyrosinase inhibition

1. Introduction

Nigella sativa L., (black cumin) a member of the Ranunculaceae family, is a blooming annual herbaceous plant. It is a tiny shrub that bears pale blue to white blooms and thin green leaves. It usually reaches a height of 20 to 90 cm. Tiny cumin-like black seeds are present in its ripe fruit. In the Middle East and some Asian nations, *N. sativa* seeds are widely used in traditional medicine to promote health. Since ancient times, *N. sativa* has been valued in a number of traditional medical systems, including Ayurveda, Islamic Prophetic, and Unani Medicine. It has been dubbed “the herb from heaven” for its use in a wide range of conditions, including asthma, bronchitis, back pain, chest congestion, cough, diabetes, dizziness, dysmenorrhea, fever, headaches, infection, infertility, inflammation, migraine, obesity, rheumatism, hypertension, and gastrointestinal disorders such as diarrhea, dysentery, dyspepsia, and flatulence [1–3]. *N. sativa* seeds and their oil exhibit a broad spectrum of pharmacological activities, supporting their traditional and modern medicinal use. The seeds have demonstrated analgesic, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, bronchodilatory, hepatoprotective, immunomodulatory, and spasmolytic effects, benefiting cardiovascular, gastrointestinal,

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neurological, and renal systems [1,4,5]. Extracts and constituents of *N. sativa* suppress coughs, dissolve kidney stones, inhibit carcinogenesis, reduce abdominal pain, alleviate diarrhea, and provide gastroprotection [6–11]. The essential oil shows anthelmintic, antimicrobial, antinematodal, antischistosomal, antiviral and effects [12–17].

The seeds of *N. sativa* contain 36–38% oil, primarily composed of linoleic (50–60%), oleic (20–23%), palmitic (12.5%), dihomolinoleic (10%), and eicosadienoic (3%) acids, along with sterols and sterol derivatives [18–20]. Important bioactive constituents include carvacrol, dithymoquinone (nigellone), *p*-cymene, terpenes, thymohydroquinone, thymol, and thymoquinone (TQ) [1]. Most pharmacological effects are attributed to nigellone and TQ which modulate oxidative stress, inflammatory mediators, MAPK, NF-κB, PPAR-γ, STAT3, apoptosis, cell cycle arrest, angiogenesis, and enhance chemotherapy efficacy. Nigellone's antiasthmatic effect is linked to inhibition of histamine release from mast cells and suppression of 5-lipoxygenase products [21–23]. In addition, *N. sativa* seeds contain alkaloids (nigellicimine nigellicine, nigellidine), saponins (e.g., α-hederin), minerals and vitamins [24,25].

A crucial analytical method for determining the fatty acid composition and bioactive components of fixed oils is Gas Chromatography (GC), which is frequently combined with mass spectrometry (GC-MS) or flame ionization detection (GC-FID). In quality control investigations of *N. sativa* oils GC techniques are essential for verifying analytical procedures. In the present study GC analysis on the cold pressing Turkish and Syrian *N. sativa* seed oils was conducted to compare their fatty acid ingredients. In addition, high-performance liquid chromatography (HPLC) was used to determine the TQ content, a key bioactive compound responsible for many of the pharmacological effects of *N. sativa.* HPLC is particularly suitable for the quantification of TQ due to its high sensitivity, reproducibility, and ability to separate thermolabile and polar constituents that are not easily detectable by GC. The combination of GC for fatty acid profiling and HPLC for TQ quantification allows for a comprehensive assessment of the phytochemical composition, ensuring accurate evaluation of the oils' chemical quality and their potential contribution to biological activities. The tyrosinase inhibitory properties of the fixed oils and TQ were also investigated in the current work.

2. Materials and Methods

2.1. Fixed Oils

N. sativa plants were cultivated in agricultural fields located in Polatlı (Ankara, Türkiye) during 2021 using seeds of Turkish and Syrian origin. Voucher specimens of each plant were deposited in the Herbarium of the Faculty of Pharmacy, Gazi University, with the numbers of GUE-4051 and GUE-4052, respectively. Fixed oils were obtained from the seeds by the cold-pressing (mechanical pressing) technique using a mechanical screw press (Karaerler NF 1000 cold press oil machine, Ankara, Türkiye) operated below 40 °C to preserve the natural composition of bioactive compounds, avoiding the use of heat or chemical solvents [26]. This method allows for the retention of thermolabile constituents, such as TQ and polyunsaturated fatty acids, while minimizing the degradation of sensitive phytochemicals. The resulting cold-pressed oils of Turkish and Syrian *N. sativa* seeds were collected, filtered to remove particulate matter, and stored in dark at 4 °C until further phytochemical and biological analyses. This approach ensures that the chemical profile of the oils reflects the native composition of the seeds, enabling an accurate assessment of their fatty acid content, TQ concentration, and potential biological activities.

2.2. Gas Chromatography-Flame Ionization Detector (GC-FID) Analysis

All chemicals used were of analytical reagent grade. HatGrup Ltd. (Ankara, Türkiye) provided helium, hydrogen, and dried air gases for Gas Chromatography (GC) with 99.99% purity. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). GC (7890A GC System, Agilent Technologies Inc., Santa Clara, CA, USA), a capillary column Rt-2560 (100 m, 0.25 mm ID, 0.2 µm) (Restek Corporation Bellefonte, Bellefonte, PA, USA), vial insert, 250 µL, glass with polymer feet, vial, screw top, 2 mL, amber and cap, screw, blue, PTFE/red silicone septa (Agilent Technologies Inc., Santa Clara, CA, USA) were used. Supelco 37 Component FAME Mix certified reference material (C4-24) (Sigma-Aldrich, St. Louis, MI, USA) was used as fatty acid metyl ester standarts (FAME37.)

2.2.1. Preparation of Fatty Acid Methyl Esters (FAMEs) Standard

The oil samples were first dried in a laboratory oven maintained at 100 °C to remove any residual moisture that could interfere with the subsequent derivatization and analysis steps. After cooling to room temperature in a desiccator, exactly 1.0 g of the dried oil was accurately weighed into a clean, dry test tube. To this, 2.0 mL of

analytical grade *n*-heptane was added as a non-polar solvent to facilitate dissolution of the oil. The mixture was shaken thoroughly until a homogeneous solution was obtained. Next, 0.2 mL of 2 M methanolic potassium hydroxide solution was introduced into the test tube to promote the transesterification of triglycerides into their corresponding FAMEs. The sample was immediately vortexed vigorously for approximately 30 s to ensure efficient contact between the oil, solvent, and reagent. Following this step, the tube was allowed to stand undisturbed until clear phase separation occurred. At this stage, the upper organic layer, consisting predominantly of the *n*-heptane phase containing the FAMEs, was carefully decanted without disturbing the lower aqueous phase. The resulting organic extract was directly subjected to gas chromatography analysis using a flame ionization detector (GC-FID) for the qualitative and quantitative determination of fatty acid methyl ester composition [27].

2.2.2. Analysis of FAMEs with GC-FID

FAME37, C4-24 standard, and fatty acids in oil samples were analyzed by GC equipped with an auto sampler model Agilent 7693, and FID. A capillary column Rt-2560 (100 m, 0.25 mm ID, 0.2 µm), vial insert, 250 µL, glass with polymer feet, vial, screw top, 2 mL, amber and cap, screw, blue, and PTFE/ red silicone septa were used as the column and sample vial, respectively. GC oven was programmed to 100 °C (4 min hold), to 240 °C at 3 °C/min (hold 15 min). The injector and FID detector temperatures were 225 °C and 250 °C, respectively. Injection volume was 1 µL with a split ratio 20:1. Helium was used as the carrier gas at 1.2 mL min^{-1}, 20 cm s^{-1} at 175 °C. H_2 flow and air flow were 30 mL/min and 300 mL/min, respectively [28,29].

FAMEs in the oil samples were identified from the chromatogram by comparing their retention times (t_R) with standard FAME37, C4-24, and the number of FAMEs in the oil samples was expressed as a percentage by weight of all FAMEs from the total detected fatty acids (Figure 1). Peak area was used for quantitative analysis of FAMEs. The results were expressed as a percentage (%wt/wt) of all fatty acids detected.

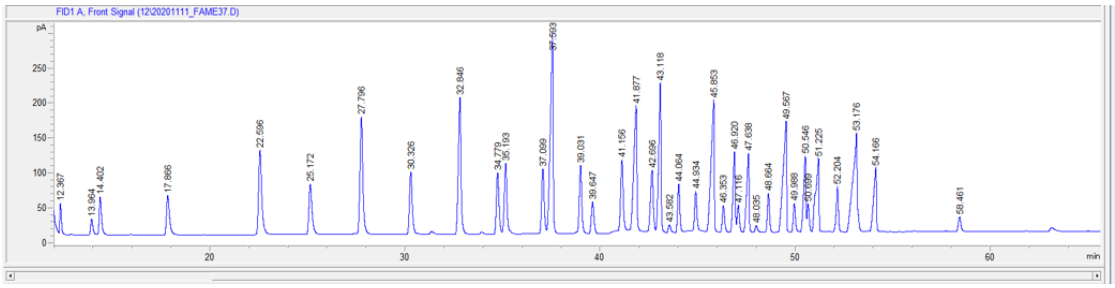

Figure 1. GC-FID chromatogram of FAME37 mix, C4-C24 reference standard material.

2.3. HPLC Analysis Conditions

Sample preparation and HPLC analysis were carried out following a previously reported method by our group with minor adjustments [30]. Briefly, 20 µL of oil sample was loaded onto a solid-phase extraction (SPE) cartridge (Agilent AccuBond II ODS-C18) and eluted with 1.5 mL of methanol prior to analysis. An equipment of Agilent Series 1200 liquid chromatograph, equipped with a binary pump system and a variable-wavelength detector system were used for the chromatographic analyses. Separations were applied on a reverse-phase Eclipse XDB-C18 column (5 µm, 4.6 mm × 15 mm, Agilent, USA) using isocratic elution with a mobile phase consisting of water-methanol-2-propanol (50:45:5, *v*/*v*/*v*) at a flow rate of 0.9 mL/min. Injection volume was set at 10 µL. Data collection was performed using ChemStation software B.02.01-SR2 (Agilent, Santa Clara, CA, USA). The identification of TQ was achieved by comparing its retention time and UV spectrum to an authentic standard, quantified by external calibration based on measurement of peak areas. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as 3.3 and 10 times the standard deviation of the response divided by the slope of the calibration curve, respectively.

2.4. Determination of Tyrosinase Inhibition Effect

The inhibition of tyrosinase was evaluated using a modified spectrophotometric method with L-DOPA (Sigma, St. Louis, Missouri, USA) as the substrate, following the procedures outlined by Masamoto et al. (1980) and Lee et al. (2009) [31,32].

For initial, 10 µL of the sample [Turkish or Syrian *N. sativa* oils or Thymoquinone (BioVision, USA)] solution or DMSO was added to a well. Then, 80 µL of 67 mM phosphate buffer (pH 6.8) and 30 µL of 5 mM L-DOPA were introduced. After a 10-min incubation at 37 °C, 30 µL of mushroom tyrosinase (Sigma, EC 1.14.18.1)

were added as the enzyme source. Following another 20 min of incubation, the absorbance at 492 nm was measured using an ELISA microplate reader (Spectramax ABS microplate reader, Sunnyvale, CA, USA). *Alpha*-kojic acid (Sigma, St. Louis, Missouri, USA) was utilized as the standard, and all experiments were conducted in triplicate.

The percentages of tyrosinase inhibition for the evaluated samples were computed using the formula:

Inhibition% = 100 − [(A_1/A_2 × 100], A_1 = Absorbance of samples at 492 nm, A_2 = Absorbance of control at 492 nm

3. Results and Disussion

Fatty acid compositions and GC-FID chromatograms of Türkiye and Syria-originated *N. sativa* seed oils were presented in Table 1 and Figure 2.

Table 1. Fatty acid compositions (mean percentage) of Türkiye and Syria-originated *N. sativa* seed oils.

Fatty Acids	% FAME, Mean ± Standard Deviation (n = 3)	
	Turkish *N. sativa* Seed Oil	Syrian *N. sativa* Seed Oil
C14:0 myristic acid	0.17 ± 0.02	0.16 ± 0.02
C16:0 palmitic acid	12.32 ± 0.06	12.49 ± 0.07
C16:1 palmitoleic acid	0.09 ± 0.01	0.24 ± 0.02
C18:0 stearic acid	3.82 ± 0.04	3.67 ± 0.04
C18:1 oleic acid	24.08 ± 0.22	24.91 ± 0.23
C18:2 linoleic acid	54.14 ± 0.45	54.39 ± 0.50
C20:0 arachidic acid	0.22 ± 0.02	0.23 ± 0.02
C20:1 cis-eicosenoic acid	0.29 ± 0.02	0.31 ± 0.02
C18:3 α-linolenic acid	0.21 ± 0.02	0.24 ± 0.02
C20:2 cis-11.14-eicosadienoic acid	2.59 ± 0.03	2.38 ± 0.05
C22:2 cis-13.16-docosadienoic acid	0.82 ± 0.01	0.11 ± 0.02
C24:0 lignoceric acid	0.03 ± 0.01	0.04 ± 0.01

The fatty acid composition of cold-pressed oils from Turkish and Syrian *N. sativa* seeds was analyzed and expressed as percentages of total fatty acids. Both oils were characterized by a high content of polyunsaturated fatty acids (PUFAs), with linoleic acid (C18:2) being the dominant component, accounting for 54.14% in the Turkish oil and 54.39% in the Syrian oil. Oleic acid (C18:1) was the second most abundant fatty acid, representing 24.08% and 24.91% of the total fatty acids in the Turkish and Syrian oils, respectively. Saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) were present at moderate levels (12–13% and 3–4%, respectively), whereas minor fatty acids including myristic (C14:0), palmitoleic (C16:1), α-linolenic (C18:3), and very long-chain fatty acids (C20:2, C22:2, C24:0) were present in trace amounts. Overall, the fatty acid profiles of both oils were highly similar, with only slight variations in minor components.

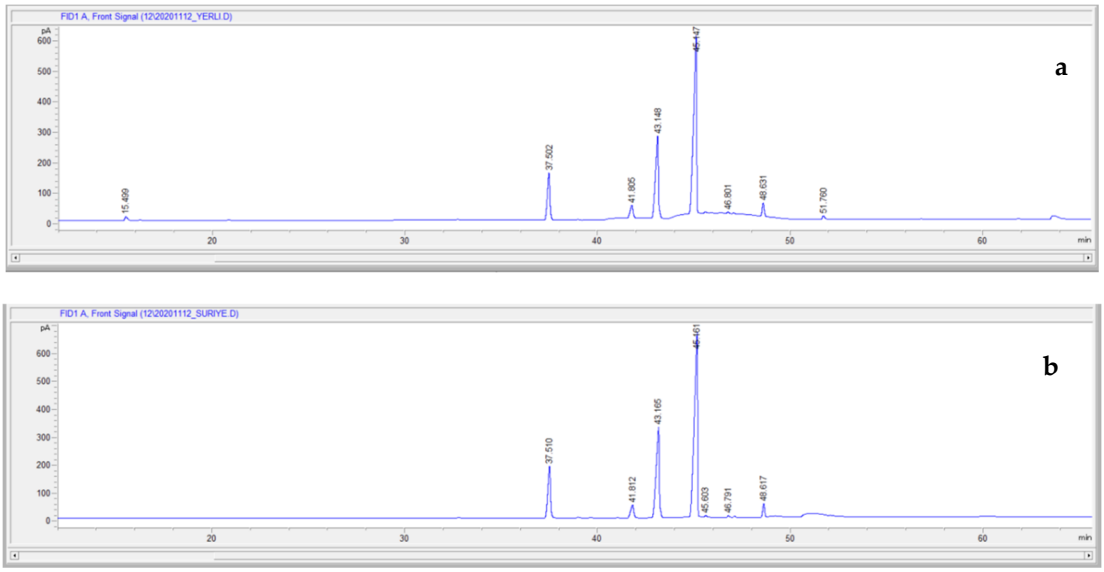

Figure 2. GC-FID chromatograms. (**a**) Turkish *N. sativa* seed oil sample; (**b**) Syrian *N. sativa* seed oil sample.

These results are consistent with previous reports on *N. sativa* seed oils, which generally indicate that linoleic acid constitutes 50–60% of the total fatty acids, followed by oleic acid (20–25%) and palmitic acid (~12%) [4,18–20]. Minor differences observed between Turkish and Syrian oils may reflect geographical and environmental influences on fatty acid biosynthesis, in agreement with previous studies that reported regional variations in *N. sativa* seed composition [20].

According to the results of HPLC analysis, the TQ level in the Turkish *N. sativa* seed oil sample was 2.31%, while it was found to be absent in the Syrian *N. sativa* seed oil sample (Table 2, Figure 3).

Table 2. Calibration data for TQ.

Linearity Range (ppm)	Calibration Equation	Correlation Coefficient	LOD (mg/mL)	LOQ (mg/mL)
0.5–500	y = 26.343x + 49.822	0.9958	0.0427	0.1292

LOD: Limit of detection; LOQ: Limit of quantification.

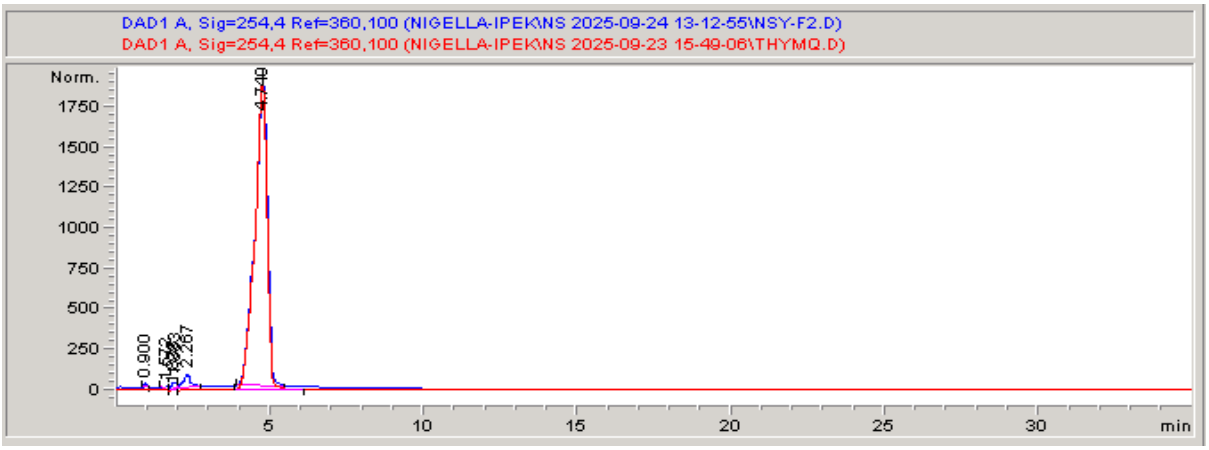

Figure 3. Overlay of chromatograms obtained from Turkish *N. sativa* seed oil sample (blue) and TQ standard (red).

Previous studies have reported a wide variation in TQ levels depending on the environmental and agronomic factors including geographical origin and cultivation conditions as well as and extraction methods [33–35]. Our finding that the Syrian oil lacked detectable TQ could therefore be linked to varietal differences, seed freshness, or post-harvest processing, as highlighted in earlier reports. On the other hand, the relatively high percentage observed in the Turkish sample aligns well with previous studies emphasizing Turkey as one of the regions yielding TQ-rich *N. sativa* oils [36,37].

Fixed oils originating from Türkiye and Syria were tested for tyrosinase inhibition at a stock concentration of 2 mg/mL, and TQ was tested at a concentration of 1 mg/mL. No inhibition was detected in the fixed oils, whereas TQ showed a low level of inhibition (10.84% ± 1.04). *Alpha*-Kojic acid, used as a positive control, inhibited the enzyme by 92.07% ± 0.12 at a stock concentration of 1 mg/mL.

Our earlier work with TQ-rich methanolic seed extracts showed potent antiproliferative and pro-apoptotic effects in pancreatic cancer cells via modulation of the NRF2/HO-1 and TNF-α signaling pathways [26]. Cold-pressed oils primarily contain non-polar components such as fatty acids and hydrophobic terpenes, whereas methanolic extracts are enriched in polar compounds, including TQ, nigellone, and phenolic constituents, which are more effective at modulating enzymatic activity and cellular signaling pathways. This finding suggests that, despite the presence of bioactive compounds, the oils in their cold-pressed form may not exert notable anti-melanogenic effects through these specific enzymatic pathways under the tested conditions. Long-chain fatty acids are nutritionally valuable but are not typically associated with direct inhibition of tyrosinase. While linoleic acid has been suggested to modulate pigmentation indirectly through effects on melanogenesis and skin barrier function [38] the absence of aromatic or phenolic structures in the oil's dominant components likely explains the lack of inhibitory activity observed in our assay. These findings agree with earlier reviews, indicating that strong tyrosinase inhibitors are usually polyphenols, flavonoids, or related aromatic compounds [39].

In contrast, TQ, a minor constituent of *N. sativa* oil, showed weak inhibition (~10.84% at 1 mg/mL). This is consistent with studies reporting that TQ suppresses melanogenesis in melanoma cells mainly through downregulation of tyrosinase expression and signaling pathways, rather than acting as a potent direct inhibitor [40]. Taken together, our results suggest that the fixed oil fraction of *N. sativa* does not contribute to tyrosinase

inhibition, and that any antimelanogenic potential of the plant is more likely attributable to minor bioactive compounds such as TQ rather than its major fatty acid constituents.

4. Conclusions

This study provides a comparative analysis of cold-pressed oils obtained from Turkish and Syrian *N. sativa* seeds, focusing on their fatty acid composition, thymoquinone content, and enzymatic inhibitory activities. GC analysis revealed that both oils are rich in unsaturated fatty acids, with linoleic acid as the major component, followed by oleic and palmitic acids, consistent with previously reported profiles for *N. sativa* oils. HPLC quantification confirmed the presence of thymoquinone, a key bioactive compound, although its concentration did not translate into significant inhibition of tyrosinase under the tested conditions. These findings suggest that while cold-pressed oils preserve essential fatty acids and thymoquinone, the concentration of polar bioactive compounds may be insufficient to exert measurable enzymatic activity *in vitro.* Nonetheless, the phytochemical richness of these oils supports their potential nutritional and pharmacological value, particularly as sources of essential fatty acids, tocopherols, and antioxidant constituents. The results underscore the importance of extraction methods and seed origin in determining the chemical composition and biological effects of *N. sativa* products. Future studies may explore alternative extraction techniques or formulations to enhance the activity of these oils.

Author Contributions

I.S.: Conceptualization, methodology, investigation, writing, reviewing and editing A.B.: Analysis, investigation, software, validation; S.A.E: Analysis, investigation, software, validation; F.S.S.D.: Analysis, investigation; M.A.: Provision of fixed oil samples. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

All data will be available as per demand.

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Conflict of Interest Disclosure

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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