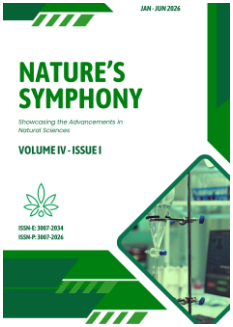
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Research Article

Characterization of *Nigella sativa* Oil–Camphor Formulation and Evaluation of Its Enhanced Anti-Inflammatory and Antimicrobial Activities

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Antioxidant activity,
Antibacterial activity

ABSTRACT

Camphor is a volatile monoterpenoid that is well known for its antimicrobial and anti-inflammatory properties, whereas *Nigella sativa* oil is a natural oil that exhibits a variety of bioactive phytochemicals. In this study, a formulation of *Nigella sativa* oil and camphor was prepared, and its physicochemical, chemical, thermal, and biological properties were evaluated with respect to the corresponding parts. The oil quality of *Nigella sativa* was found to be satisfactory, as indicated by its fat content of 40.21%, ash content of 6.74%, peroxide value of 3.995 ± 0.05 mEq/kg, free fatty acid value of 0.647 ± 0.001, iodine value of 124, and saponification value of 189.37. Numerous substantial bioactive compounds, such as camphor (8.78 min), p-cymene (4.12 min), thymoquinone (10.78 min), carvacrol (12.36 min), and longifolene (15.32 min), were identified by GC–MS profiling. The appropriate incorporation of camphor into the oil phase was demonstrated by determining the chemical composition of both the oil and the camphor in the resulting formulation. Characteristic absorption bands associated with C–H stretching at about 2922 and 2853 cm^{-1}, C=O stretching around 1744 cm^{-1}, C=C stretching near 1655 cm^{-1}, and C–O stretching within the 1238–1162 cm^{-1} range were identified by FTIR analysis. These spectral features suggested there was relatively low chemical interaction and indicated that the individual elements were generally physically incorporated. A couple of contrasting stages of deterioration were observed by thermal analysis via TGA: camphor volatilization commenced between 80 and 180°C, and the oil matrix decomposed between 250 and 450°C. Moreover, the biological activity of the combined formulation was higher than that of each component. In addition to higher antibacterial inhibition zones, DPPH radical-scavenging activity reached 94.73 ± 1.24% at 1000 ppm, and anti-inflammatory activity was approximately 90.12% at 50 µL. Overall, the findings support the formulation potential as an organic bioactive preparation for further investigation by demonstrating that the addition of camphor could improve a variety of chemical and biological attributes of *Nigella sativa oil*.

1. INTRODUCTION

For a long time, *Nigella sativa*, also known as black seed or black cumin, has been prized for its therapeutic properties. It is indigenous to parts of North Africa, the Middle East, and Southwest Asia, where its seeds have been used for centuries for various kinds of medical applications (Shabana *et al.*, 2013). Multiple physiologically active substances found in the seeds gained substantial scientific interest owing to their diverse therapeutic effects. The relevance of thymoquinone (TQ), along with alkaloids and saponins, which have been proposed to contribute significantly to the medicinal potential of the plant in the management of chronic disorders, has been highlighted in recent studies (Alberts *et al.*, 2024). The central defense mechanism of the immune system against toxic stimuli and damaged tissues is inflammation. However, if the inflammatory response persists for an extended period of time, inflammation may have an important role in the onset and progression of a variety of chronic disorders, such as cancer, autoimmune diseases, and cardiovascular disorders (Chen *et al.*, 2018).

The anti-inflammatory properties of *N. sativa* have been well documented in the literature. The fundamental bioactive components, specifically thymol and thymoquinone, exhibited the ability to affect inflammatory signaling pathways along with regulating cytokine release (Shafodino *et al.*, 2022). Studies have reported that *N. sativa* extracts can inhibit the production of pro-inflammatory cytokines as well as enzymes involved in inflammatory responses. In addition, recent research suggests that thymoquinone can suppress pyroptosis, an inflammatory type of programmed cell death, while limiting the accumulation of reactive oxygen species (ROS) by regulating the SIRT1/NF-κB signaling pathway (Alberts *et al.*, 2024). These activities may contribute to the reduction of prolonged inflammation and could be beneficial in conditions associated with chronic inflammatory responses (Ojueromi *et al.*, 2022). Through its effects on several pathways involved in inflammation, *N. sativa* therefore has potential as a natural complementary option to conventional anti-inflammatory treatments (Yimer *et al.*, 2019).

The growing problem of antibiotic resistance and the need for new antimicrobial compounds have led to increased interest in natural products as possible sources of alternative agents. *N. sativa* has attracted particular attention due to its long-standing traditional use against infections and its reported antimicrobial effects against a broad range of microorganisms (Forouzanfar *et al.*, 2014). A number of studies have examined its activity against bacterial, viral, and fungal pathogens. More recent research has also focused on its effectiveness against antibiotic-resistant organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and various mycobacterial species. The antimicrobial effects of *N. sativa* have been associated with its bioactive constituents, which may disrupt microbial cell membranes and interfere with important cellular and metabolic processes (Alberts *et al.*, 2024).

N. sativa seed oil is considered a beneficial edible oil due to its nutritional value and a range of biological properties that may contribute to human health (Al-Attass *et al.*, 2016). Various studies have reported its antioxidant, anti-inflammatory, antibacterial, antitumor, and immunostimulatory activities. Traditionally, the oil has also been used for managing respiratory conditions such as bronchitis, asthma, and emphysema (Ezzat Abd El-Hack *et al.*, 2016). *N. sativa* seed oil has become more popular due to growing evidence of its advantageous qualities, especially in Middle Eastern nations (Arshad *et al.*, 2025).

Camphor is a transparent, wax-like material with a distinct odor. It is usually produced commercially from turpentine oil or extracted from the wood of *Cinnamomum camphora* (Cai *et al.*, 2021). The various biological roles of this terpenoid compound, including its antibacterial, antiviral, and anti-inflammatory characteristics, have attracted attention (Fazmiya *et al.*, 2022). Recent research on *C. camphora* demonstrated that its essential oils, notably those that comprise camphor and borneol, may diminish pro-inflammatory cytokines that involve TNF-α, IL-1β, and IL-6 in a concentration-dependent way (Alberts *et al.*, 2024). In light of its distinctive odor and therapeutic attributes, camphor is often used in pharmaceutical, cosmetic, and insect repellent products, also owing to its traditional health benefits (Shah *et al.*, 2026).

The influence of garlic, onion, and cinnamon extracts on the properties of *N. sativa* oil has already been investigated; meanwhile, the addition of camphor to *N. sativa* oil has received little attention. Accordingly, the objective of the present study was to create a formulation of *N. sativa* oil and camphor and examine its physicochemical characteristics, chemical composition using GC-MS and FTIR, and thermal response by TGA. Moreover, the antibacterial, anti-inflammatory, and antioxidant properties of the formulation were evaluated and contrasted with those of camphor and *N. sativa* oil alone. The objective of the study was to investigate the extent to which introducing camphor could improve the biological and functional properties associated with *N. sativa* oil.

2. METHODOLOGY

Before developing the formulation, the investigation began with the physicochemical characterization of *N. sativa* oil. The oil extraction yield, ash content, saponification value, iodine value, and thermal stability were subsequently determined by applying traditional analytical approaches. To identify the quality and structural properties of the extracted oil and *N. sativa* seeds, these parameters were determined. The oil–camphor solution was created by adding camphor to the extracted *N. sativa* oil after the initial characterization. Consequently, to determine the integration of camphor and identify the overall chemical composition of the obtained product, the final composition was evaluated using both spectroscopic and chromatographic techniques, particularly FTIR and GC-MS.

2.1 Extraction of *Nigella sativa* Oil

N. sativa seed oil was extracted using a Soxhlet apparatus following the method reported by (Danlami *et al.*, 2014). Briefly, 40 g of crushed *N. sativa* seeds were extracted with 150 mL of hexane for 16 h. After extraction, the obtained oil was collected and stored in dark-colored bottles at −20 °C until further analysis. The stored oil was used for physicochemical characterization as well as antioxidant, anti-inflammatory, and antibacterial assays. All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.2 Preparation of *Nigella sativa* Oil-Camphor Formulation

The *N. sativa* oil–camphor formulation was prepared by adding finely powdered camphor to *N. sativa* oil at a concentration of 4.8% w/w, corresponding to 0.48 g of camphor in 10.35 mL of oil. Camphor was gradually added to the oil with continuous stirring at room temperature for 20–30 min until a uniform mixture was obtained. The mixture was gently warmed to 35–40 °C to improve the dispersion of camphor, while higher temperatures were avoided because of the volatile nature of camphor. The determined concentration was below the suggested maximum level for topical application and in the topical concentration range for camphor described in previous

studies. Despite the physicochemical, spectroscopic, thermal, and biological examinations, this ratio still remained constant. During preparation, the mixture was placed in an opaque glass container and allowed to be stored at room temperature until thermal, GC-MS, and FTIR analyses were performed.

2.3 Physicochemical Properties of *Nigella sativa* Oil

2.3.1 Oil Extraction Yield

N. sativa seed oil output was computed following the method given by Maxwell (1984). Briefly, the oil was extracted from the seed material using hexane, followed by removal of the solvent to recover the extracted oil. The amount of oil obtained was measured by weight, and the extraction yield was calculated as a percentage of the original weight of the seed sample.

2.3.2 Ash Content

Approximately 0.5 g of *N. sativa* oil was placed in a muffle furnace and heated at 550 °C for 12 h. After incineration, the remaining ash was weighed, and the total ash content was calculated as a percentage of the original sample weight according to the method reported by (Khoddami *et al.*, 2011).

2.3.3 Saponification Value

N. sativa oil (1 g) was taken in a pre-weighed beaker and dissolved in 3 mL of fat solvent. The contents were transferred to a 250 ml conical flask by rinsing the beaker with additional solvent. To the flask, 25 ml of 0.5 mol/l alcoholic KOH was added, and the mixture was refluxed. A blank flask, containing everything except the fat, was also set up. Both flasks were heated in a boiling water bath for 30 min. After cooling to room temperature, titrations were performed with 0.5 mol/l HCl and phenolphthalein indicator. The difference between the blank and test readings yielded the volume (mm) of 0.5 mol/l KOH required to saponify 1 g of fat. The saponification value was calculated using the formula provided by (Alrashidi *et al.*, 2020).

2.3.4 Iodine Value

The iodine value was calculated using the method described by (Alrashidi *et al.*, 2020). A fat solution (10 ml) was mixed with ICL solution (25 ml) (iodine monochloride solution in glacial acetic acid) in a stoppered bottle and left in the dark for 1 h after thorough shaking. A blank was prepared with chloroform (10 ml) in place of the fat solution. KI solution (10 ml) was added, and the liberated iodine was titrated with standard thiosulfate. The difference between the blank and test readings provided the volume (mm) of 0.1 mol/l thiosulphate required to react with the equivalent volume of iodine.

2.4 Characterization of *Nigella sativa* Oil, Camphor and their Formulation

2.4.1 GC–MS-Based Chemical Profiling

GC–MS analysis was carried out to identify the volatile constituents present in camphor, *N. sativa* oil and their formulation. The samples were diluted in GC-grade n-hexane and filtered prior to analysis. Separation was performed using a GC–MS system equipped with an HP-5MS capillary column using helium as the carrier gas at a flow rate of 1.0 mL/min. A 1 µL sample was injected in split mode. The oven temperature was programmed from 50°C to 280°C. The mass spectra were recorded under electron ionization mode at 70 eV within an m/z range of 40–500. The obtained spectra were compared with NIST library data for compound identification (Benkaci–Ali *et al.*, 2007).

2.4.2 FTIR Spectroscopic Analysis

FTIR analysis was performed separately for *N. sativa* oil, camphor and the prepared *N. sativa* oil–camphor formulation in order to evaluate their characteristic functional groups and

possible interactions after formulation. Initially, the FTIR spectrum of pure *N. sativa* oil was recorded to identify the characteristic bands of triglycerides, unsaturated fatty acids and ester functional groups. Similarly, pure camphor was analyzed individually to confirm the presence of characteristic ketonic and bicyclic monoterpene functional groups. Afterward, camphor was mixed with *N. sativa* oil to prepare the formulation, and the FTIR spectrum of the formulation was recorded under the same conditions. To evaluate the incorporation of camphor into the *N. sativa* oil matrix, and identify any shifts in the distinctive absorption bands, a comparative FTIR analysis was conducted. There were no significant additional peaks in the formulation spectrum; only the most prominent peaks associated with camphor and *N. sativa* oil were observed. This pattern suggests that the two components were physically combined without undergoing any major chemical reaction (Rohman and Ariani, 2013).

2.4.3 Thermogravimetric Analysis

Thermogravimetric analysis was performed to evaluate the thermal behavior of camphor, *N. sativa* oil and the prepared *N. sativa* oil–camphor formulation. Approximately 5–10 mg of each sample was placed in an alumina crucible and heated from room temperature to 600°C at a heating rate of 10°C/min under a nitrogen atmosphere. The continuous change in sample weight was recorded as a function of temperature.

The onset degradation temperature, major weight-loss stages and final residual mass were determined from the TGA curves. Pure camphor was analyzed to observe its volatilization/sublimation behavior, while *N. sativa* oil was analyzed to determine the thermal degradation profile of triglycerides and fatty acid constituents. The formulation was analyzed under the same conditions to evaluate the effect of camphor incorporation on the thermal stability of the oil-based formulation. A two-stage weight-loss pattern in the formulation was considered indicative of camphor volatilization followed by degradation of the oil matrix (Mohammed *et al.*, 2016).

2.5 Biological Evaluation of *Nigella sativa* Oil and Camphor and their Formulation

2.5.1 Antioxidant Activity

The DPPH radical scavenging assay was performed following the method described by Blois (1958) and Brand-Williams *et al.* (1995), with minor modifications, and ascorbic acid was used as the standard antioxidant. The antioxidant activity of camphor, *N. sativa* oil, and the camphor–*N. sativa* oil formulation was evaluated using the modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Serial concentrations of the test samples were prepared at 1000, 500, 250, 125, 62.5, 31.25, 15.62, and 7.81 ppm. Camphor was dissolved in DMSO, while *N. sativa* oil was prepared in methanol. Ascorbic acid was used as the standard antioxidant and was prepared at the same concentration range. For each concentration, 0.2 mL of sample or standard solution was mixed with 3.8 mL of 500 ppm DPPH solution. The reaction mixtures were incubated for 30 min in the dark at room temperature. Following incubation, the absorbance of the samples was recorded at 516 nm with a UV–Visible spectrophotometer. A DPPH blank prepared in ethanol was used as the control.

The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\text{Inhibition (\%)} = A0 - A1/A0 \times 100$$

Where, A_0 is absorbance of DPPH control/blank and A_1 is absorbance of sample + DPPH

The radical scavenging activity was further expressed as IC_{50}, which represents the

concentration of sample required to inhibit 50% of DPPH radicals. Lower IC_{50} values indicate stronger antioxidant activity. IC_{50} values were calculated from the inhibition percentage versus concentration curve using non-linear regression analysis.

2.5.2 Anti-Inflammatory Activity

The Anti-inflammatory activity of *N. sativa* oil, camphor, and their combined formulation was evaluated using the bovine serum albumin (BSA) protein denaturation assay as an *in vitro* surrogate model for anti-inflammatory activity, as described by Brown and Mackey (1968) and later modified by Chandra *et al.* (2012), using diclofenac sodium as the reference standard. This assay was used to estimate the ability of the samples to inhibit heat-induced BSA denaturation, which indicates their potential anti-inflammatory activity.

Different volumes of *N. sativa* oil, camphor, and *N. sativa* oil–camphor formulation were prepared in DMSO at 10, 20, 30, 40, and 50 µL concentrations. Each test sample was mixed separately with 2 mL of 1% BSA solution in test tubes. Distilled water was then added in corresponding volumes of 390, 380, 370, 360, and 350 µL, respectively, to maintain the final reaction volume. For the control, 2 mL of DMSO was mixed with 2 mL of 1% BSA solution. Diclofenac sodium was used as the standard drug and was prepared at the same concentrations of 10, 20, 30, 40, and 50 µL. All test tubes were kept at room temperature for 10 min and then incubated in a water bath at 55°C for 10 min. After incubation, absorbance was measured at 660 nm using a UV–Visible spectrophotometer. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ of inhibition} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

2.5.3 Antibacterial Activity

The broth dilution assay was performed in accordance with Wiegand *et al.* (2008), with minor adjustments, and agar disk diffusion method was used to evaluate antibacterial activity according to Bauer *et al.* (1966). Tryptone, sodium chloride, and agar were dissolved in distilled water to produce LB agar for the disk diffusion test. The mixture was subsequently heated until a homogeneous, transparent medium was obtained. After that, the medium was sterilized and transferred to aseptic Petri dishes. To allow the agar time to firm up, the plates were placed at room temperature for about 20 min. The obtained bacterial culture was transferred into an Eppendorf tube containing 1 mL of LB broth and incubated for 24 h to prepare the bacterial inoculum. Before being used in the antibacterial assay, the bacterial suspension was centrifuged for two min post-incubation. After discarding the supernatant, 100 µL of the leftover bacterial solution was spread onto LB agar plates. To facilitate the growth of bacterial colonies, the plates were incubated for a whole day. After that, a selected bacterial colony was inoculated into 200 mL of LB broth and incubated with shaking for 24 h to produce an actively growing bacterial culture. 0.1 mL of *N. sativa* oil and 10 mL of n-hexane were mixed to create the oil for the antibacterial assay. By dissolving 0.1 g of camphor in 10 mL of DMSO, a camphor stock solution was produced. As stated in Section 2.2, 0.1 mL of the previously prepared *N. sativa* oil–camphor formulation containing 4.8% w/w camphor was diluted with 10 mL of n-hexane for the combined sample. *N. sativa oil*, camphor, and the resulting formulation were subsequently diluted from the stock solutions to obtain working concentrations of 0.1, 0.2, 0.3, and 0.4 mg/mL. Sterile filter paper disks were saturated with these concentrations to assess their antibacterial properties. The sample-loaded

filter paper disks were placed on the inoculated agar surface after the bacterial suspension had already been evenly distributed over LB agar plates. Erythromycin was the standard antibacterial drug, and plates of solvent alone served as negative controls. The plates were incubated for 24 h, after which antibacterial activity was evaluated by measuring the diameter of the inhibition zones around the disks.

2.5.4 Broth Serial Dilution Assay

The broth dilution method was performed to further evaluate the antibacterial effect of *N. sativa* oil, camphor, and their combined formulation. Twelve labeled test tubes containing different sample volumes/concentrations ranging from 0.1 to 1.0 mL were prepared. Control and standard tubes were also included. Bacterial inoculum and LB broth were added to each tube, and the tubes were incubated on a shaker for 24 h.

After incubation, bacterial growth was assessed by measuring the optical density at 600 nm using a UV–Visible spectrophotometer (UV1100). Samples showing lower absorbance than untreated control were considered to have inhibited bacterial growth. Antibacterial activity was therefore evaluated from the reduction in turbidity and the corresponding decrease in absorbance at 600 nm.

2.6 Statistical Analysis

All results were reported as mean ± standard deviation based on three independent replicates. One-way analysis of variance (ANOVA) was employed to statistically assess the data, and SPSS software version 12.0 was chosen for the statistical analysis.

3. RESULTS

3.1 Physicochemical Properties of *Nigella sativa* Oil

The extraction yield, ash content, peroxide value, free fatty acid (FFA) value, saponification value, and iodine value of *N. sativa* oil are all shown in Fig 1. The ash content of the seed oil reached 6.74%, while the extraction yield was 40.21%. The FFA value, expressed as lauric acid, was 0.647 ± 0.001, and the peroxide value was 3.995 ± 0.05 mEq/kg, based on the results obtained. The iodine and saponification levels were found to be 124 and 189.37, respectively.

3.2 GC-MS Analysis

GC–MS was employed to investigate the volatile chemical profiles of camphor, *N. sativa* oil, and the resultant oil–camphor mixture. The resulting chromatograms are illustrated in Fig 2 (a–c). Differences in the dosage, distribution pattern, and relative intensity of the volatile chemicals found were represented in the distinctive chromatographic profiles that each sample produced. As a result, the chromatograms showed distinct differences in the chemical composition of camphor, *N. sativa* oil, and their combination. The GC–MS chromatogram of camphor (Fig 2a) demonstrated a relatively basic profile, with a prominent peak that was associated with camphor estimated to be 8.78 min. At retention times of approximately 3.60 and 10.12 min, respectively, smaller peaks corresponding to eucalyptol and borneol were identified. The sample was predominantly made up of this bicyclic monoterpene ketone, as indicated by the strong camphor peak. The relatively simple composition of camphor in contrast to *N. sativa* oil is further demonstrated in the relatively small number of peaks. The chromatogram of *N. sativa* oil (Fig 2b) showed a significantly more complicated profile, with numerous distinct peaks appearing throughout the chromatographic run. Volatile monoterpenes like α-thujene and p-cymene were among the majority of the compounds that eluted at shorter retention times; they were identified at approximately 2.36 and 4.12 min, respectively. One of the key bioactive compounds of *N. sativa* oil, thymoquinone, was identified as the

source of a prominent peak that appeared at approximately 10.78 min. Longifolene was observed at around 15.32 min, whereas carvacrol appeared at 12.36 min.

Conversely, the *N. sativa* oil and camphor mixture chromatogram (c) exhibited the combined chromatographic properties of the two separate components. With the exception of the primary identified *N. sativa* oil peaks, such as p-cymene, thymoquinone, carvacrol, and longifolene, the formulation maintained the distinctive characteristics of the camphor peak at approximately 8.78 min. The coexistence of these components demonstrates the integration of camphor into the *N. sativa* oil matrix. There were no obvious new peaks in the formulation chromatogram, indicating that camphor and the oil components did not interact chemically during synthesis. Further, the chromatographic pattern showed that the two systems were physically combined without losing their characteristic volatile constituents. The mixed chromatogram appeared less complex than pure *N. sativa* oil, but it displayed a higher overall peak density than camphor alone due to the overwhelming impact of camphor and thymoquinone peaks. The addition of camphor had no effect on the bioactive characteristics of *N. sativa* oil, as indicated by the thymoquinone peak reactivity. The mixture of monoterpenes, ketones, and sesquiterpenes could be responsible for the biological formulation and therapeutic attributes.

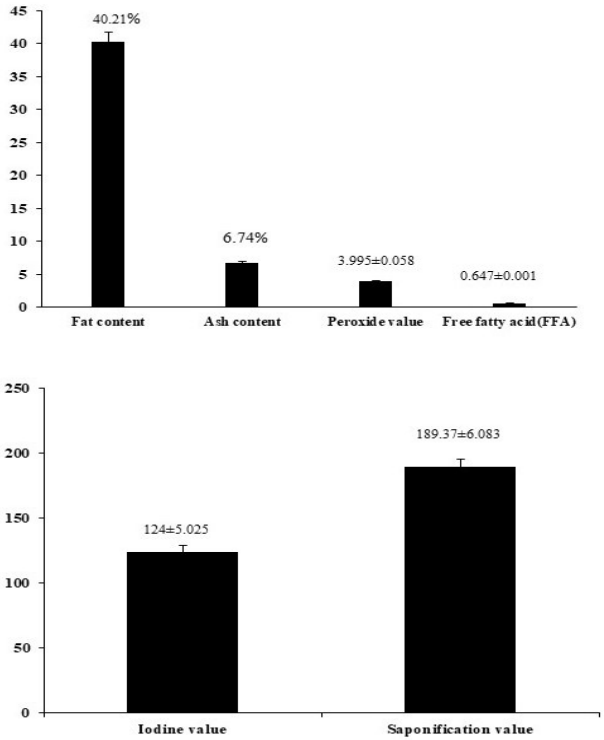


Figure 1. Physicochemical properties of *N. sativa*, including its fat, ash, peroxide, free fatty acid (FFA), iodine, and saponification levels. The findings indicate the general stability and quality of the oil with a high lipid content, low FFA level, moderate ash content, and a substantial amount of unsaturation.

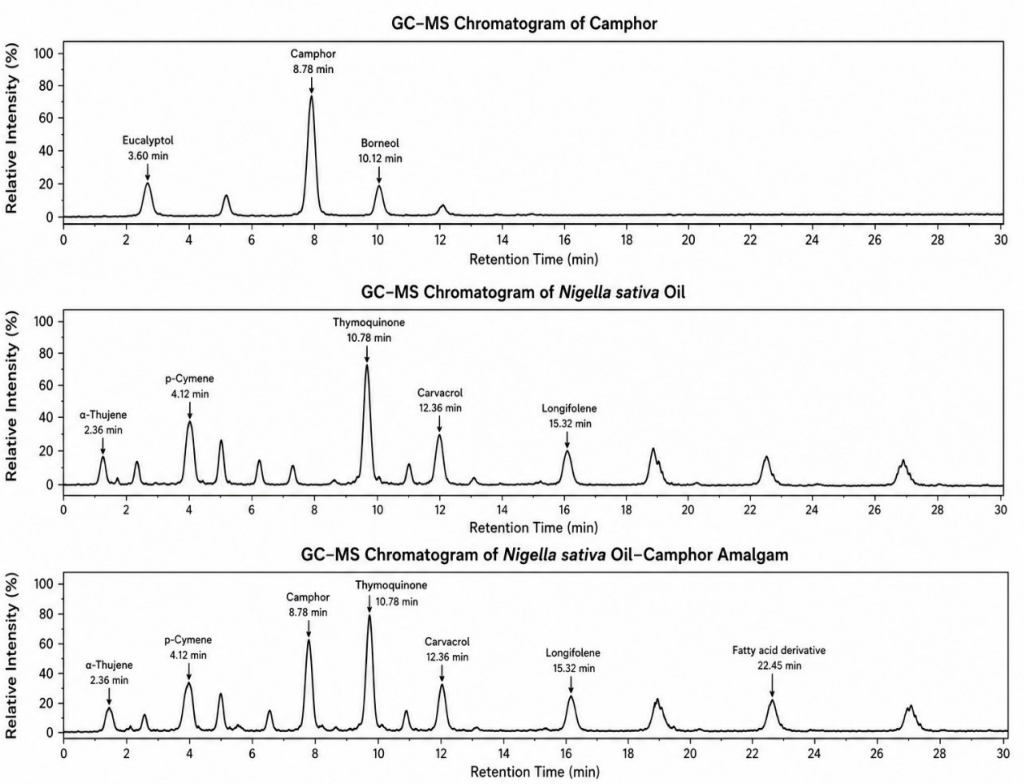


Figure 2. GC-MS chromatograms of *N. sativa* oil (middle), camphor (top), and the mixture of *N. sativa* oil and camphor (bottom). The main volatile compounds present in each sample, such as p-cymene, thymoquinone, carvacrol, and longifolene in *N. sativa* oil, eucalyptol and borneol in camphor, are illustrated by the chromatographic patterns. The formulation, which had peaks corresponding to elements derived from both components, indicated the incorporation of camphor into the oil matrix.

3.3 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of camphor, *N. sativa* oil, and the produced mixture are displayed in Fig 3 (a–c), indicating their distinct functional groups and their linkages. The spectra of pure *N. sativa* oil (Fig 3a) showed absorption bands primarily linked to substances consisting of lipids and esters. Olefinic (=C–H) stretching of unsaturated fatty acids was observed as a weak band at about 3005 cm^{-1}. Asymmetric and symmetric C–H stretching vibrations of aliphatic hydrocarbon chains were linked to the strong bands observed at about 2920 and 2850 cm^{-1}. Ester carbonyl (C=O) stretching of triglycerides, characteristic of fatty acid ester components present in the oil, was identified as the reason for substantial absorption at approximately 1740 cm^{-1}. The C=C stretching of unsaturated fatty acids was associated with the band around 1650 cm^{-1}. CH_2 bending and CH_3 deformation vibrations were associated with absorptions at about 1460 and 1375 cm^{-1}, accordingly. The absorption coefficient near 720 cm^{-1} was attributed to CH_2 rocking vibrations in long hydrocarbon chains, whereas the bands observed between 1230 and 1160 cm^{-1} were associated with C–O stretching in ester groups.

The bands observed in camphor's FTIR spectrum (Fig 3b) indicated its bicyclic monoterpene structure and ketone group. Aliphatic C–H stretching vibrations were associated with absorption at about 2950 cm^{-1}. The presence of the ketone carbonyl (C=O) group, a hallmark of camphor, was confirmed by a strong, sharp band at about 1745 cm^{-1}. Vibrations associated with CH bending and methyl deformation were detected at around 1450 and 1370 cm^{-1}, respectively. Skeletal and ring vibrations arising from camphor's bicyclic structure were associated with additional bands in the fingerprint region between 1240 and 1100 cm^{-1}. The key absorption bands associated with both starting materials were retained in the FTIR spectrum of the *N. sativa* oil–camphor formulation (Fig 3c), indicating that their basic structural features were maintained during formulation. The cumulative contributions of the ketone carbonyl group of camphor and the ester carbonyl group of *N. sativa* oil resulted in a broad, strong band at approximately 1744 cm^{-1}. Weak interactions between camphor and the oil constituents may be suggested by the band's minor broadening and slight change in relation to its separate components.

Due to the overlapping effects from the hydrocarbon chains of the oil and camphor, the standard C–H stretching region also exhibited increased intensity and broadening. Asymmetric and symmetric CH_2 stretching vibrations were recognized as the bands at approximately 2922 and 2853 cm^{-1}, correspondingly. When camphor was added, the absorbance band of the standard formulation at 1655 cm^{-1} was still discernible, indicating that the unsaturated fatty acid structures in the oil remained in their normal state. Additional peaks observed at 1463 cm^{-1}, 1378 cm^{-1}, 1238 cm^{-1}, 1162 cm^{-1}, and 722 cm^{-1} corresponded to CH_2 bending, CH_3 deformation, ester C–O stretching, triglyceride-related C–O stretching, and long-chain hydrocarbon rocking vibrations.

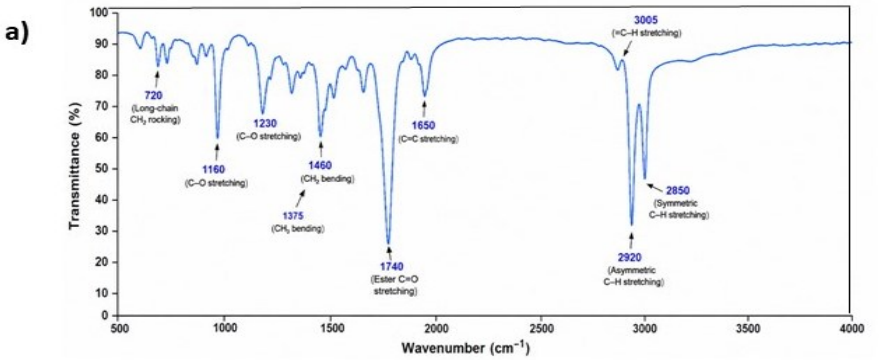
The primary distinct peaks of each component persisted, and the FTIR spectra of the formulation showed no notable additional absorption bands. This suggests that during the formulation process, there was no discernible chemical interaction or degradation between camphor and *N. sativa* oil. The modest expansion and slight changes observed in some absorption bands may be due to intermolecular interactions and the physical dispersion of camphor within the oil matrix rather than the emergence of new chemical interactions.

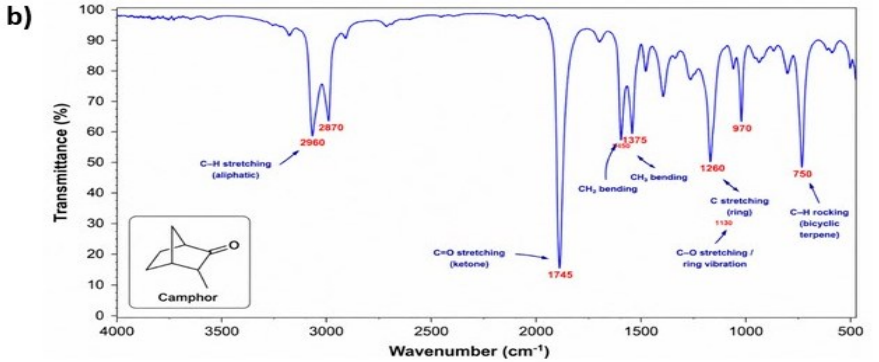
Ultimately, the FTIR results suggested that the distinctive functional groups of both compounds did not change when camphor was introduced to *N. sativa* oil. The observed broad spectrum changes indicate that the formulation arose primarily through physical mixing, indicating excellent compatibility and stability of the produced formulation despite only moderate interactions among the components.

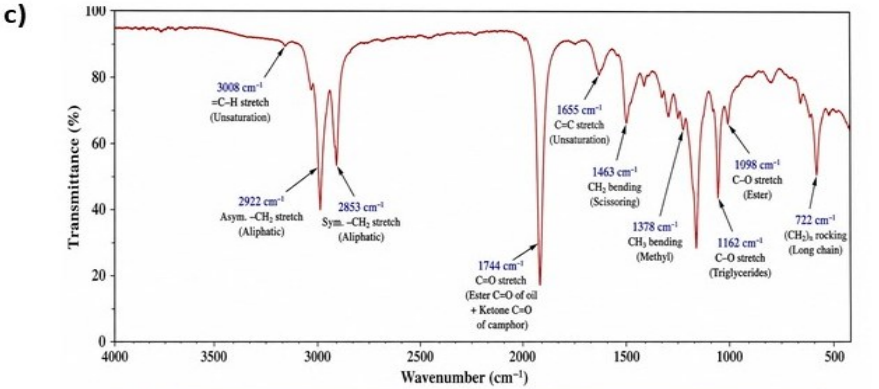
3.4 Thermogravimetric Analysis (TGA)

The temperature stability and degradation behavior of camphor, *N. sativa oil*, and the *N. sativa* oil and camphor mixture were evaluated using thermogravimetric analysis (TGA), as shown in Fig 4.

Between 80 and 180 °C, the mass of pure camphor drastically dropped. The volatilization and sublimation of camphor are the main reasons for this weight loss. As camphor is highly volatile, at 200 °C almost all of the sample mass had been lost, leaving only a slight trace. *N. sativa* oil showed noticeably greater heat stability in its TGA analysis.







Peak (cm⁻¹)	Assignment	Origin	Peak (cm⁻¹)	Assignment	Origin
3008	=C–H stretching	Nigella oil	1463	CH_2 bending (scissoring)	Oil + Camphor
2922	Asymmetric –CH_2 stretching	Oil + Camphor	1378	CH_3 bending (methyl)	Camphor / Oil
2853	Symmetric –CH_2 stretching	Oil + Camphor	1238	C–O stretching (ester)	Nigella oil
1744	C=O stretching	Oil (ester) + Camphor (ketone)	1162	C–O stretching (triglycerides)	Nigella oil
1655	C=C stretching	Nigella oil	722	$(CH_2)_n$ rocking (long chain)	Nigella oil

Figure 3. FTIR spectra of *N. sativa* oil (a), camphor (b), and the mixture of *N. sativa* oil and camphor (c). Along with the characteristic absorption bands associated with C–H, C=O, C=C, and CH vibrations, the spectra show C–O stretching bands. The spectrum (c) still shows the main characteristic peaks of *N. sativa* oil and camphor, indicating their physical absorption despite considerable chemical modification

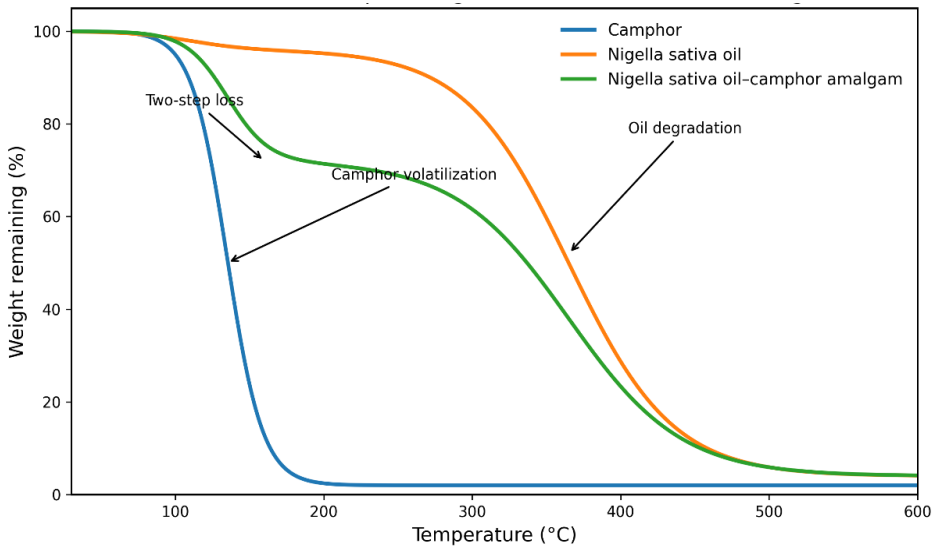


Figure 4. Thermogravimetric analysis of *N. sativa* oil, camphor, and mixture of Camphor and *N. sativa* oil

Below 150 °C, a slight decrease in weight was noticed, which may be linked to the elimination of humidity and other volatile compounds with low boiling points. The first breakdown occurred between 250 and 450 °C and was correlated with the triglyceride and fatty acid elements of the oil. There was about 5–8% residual mass left after the most major degradation stage, indicating that the oil contained more heat-resistant components. The mixture of camphor and *N. sativa* oil induced two different phases of weight loss. The decomposition of the oil matrix was associated with the second stage, which occurred between 250 and 450 °C, whereas the first phase, which took place between 80 and 180 °C, was primarily attributed to the volatilization of camphor. The presence of these distinct degradation zones suggests that camphor remained physically integrated into the oil and that each component mostly kept its own distinct thermal behavior, despite the lack of any apparent evidence of a significant chemical interaction between the components.

3.5 Biological Evaluation of *Nigella sativa* Oil, Camphor, and Their Formulation

3.5.1 Antioxidant Activity

The ability of camphor, *N. sativa* oil, and the combined formulation to scavenge DPPH radicals is shown in Fig 5 along with the corresponding IC_{50} values. Radical scavenging activity gradually increased in all samples as the concentration increased, suggesting a dose-dependent response.

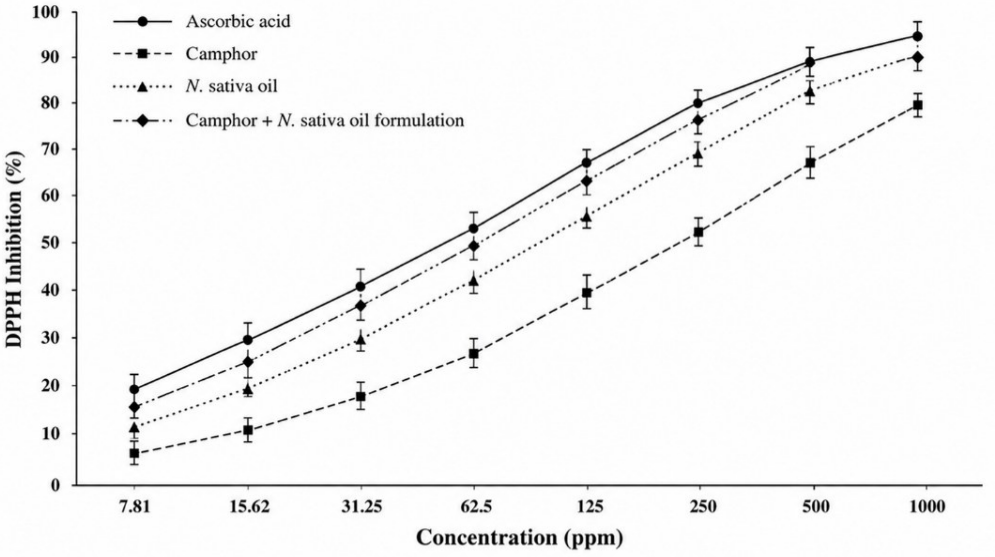
With an inhibition of 95.84 ± 1.31%, ascorbic acid demonstrated the highest scavenging activity at 1000 ppm. Among the evaluated samples, camphor exhibited the least action, with a 79.26 ± 1.26% inhibition at the same concentration, suggesting a slight potential for neutralization of DPPH radicals. *N. sativa* oil had much more efficacy than camphor, with an inhibition of 91.68 ± 1.28% at 1000 ppm. The antioxidant properties of oil may be connected to its bioactive constituents, which include thymoquinone, thymol, carvacrol, and other phenolic compounds. Both the *N. sativa* oil and camphor combination had scavenging

activity that was comparable to ascorbic acid and better than that of camphor itself. At 1000 ppm, the formulation showed 94.73 ± 1.24% inhibition, demonstrating that the addition of camphor improved the capacity of oil to scavenge radicals. The combination of camphor and the naturally occurring antioxidant substances in *N. sativa* oil may be crucial for this beneficial effect.

The differences in antioxidant activity between the samples were further verified by the IC_{50} values, while a lower IC_{50} value indicates a higher capacity to scavenge radicals. In line with its weaker antioxidant activity, camphor displayed the highest IC_{50} value. Stronger antioxidant activity was determined by *N. sativa* oil's lower IC_{50} value, and the combined formulation exhibited even greater improvement. The relatively high antioxidant activity of the formulated oil was supported by its IC_{50} value, which was around that of ascorbic acid.

3.5.2 Anti-inflammatory Activity

The percentage inhibition of BSA protein denaturation, which is used as an *in vitro* biomarker of the anti-inflammatory potential of camphor, *N. sativa* oil, and their combination is illustrated in Fig 6. When the tested volume increased progressively from 10 to 50 µL, all three samples showed a progressive increase in inhibition.



Sample	IC_{50} (ppm)	Antioxidant strength
Ascorbic acid	55.78	Very strong
Camphor	226.19	Moderate
Nigella sativa oil	97.42	Strong
Camphor + *N.* sativa oil formulation	60.51	Very strong

Figure 5. Ascorbic acid, camphor, *N. sativa* oil, and the camphor *N. sativa* oil formulation at various dosages all showed DPPH radical scavenging activity. The table presents the relevant IC_{20} values for assessing the antioxidant activities of the tested samples, and the line graph illustrates the change in percentage inhibition with increasing concentration

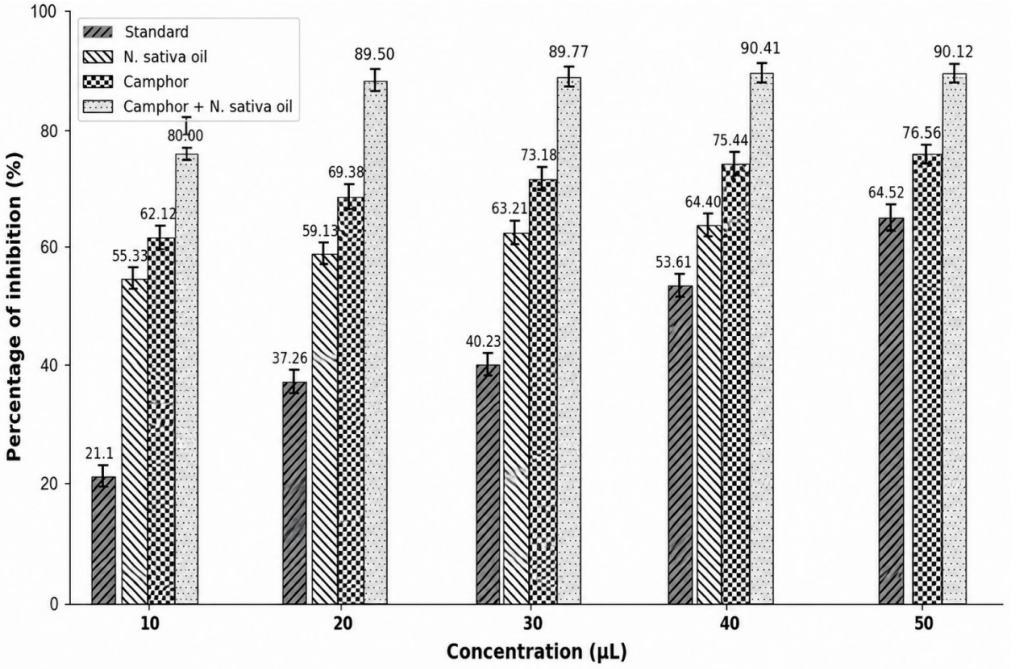


Figure 6. Percentage inhibition of BSA protein denaturation by standard, *N. sativa* oil, camphor, and camphor-*N. sativa* oil formulation at different concentrations (10–50 µL), used as an *in vitro* indicator of anti-inflammatory potential

N. sativa oil, camphor, and the combination formulation produced inhibition values of 55.24%, 62.68%, and 80.24%, respectively, at the lowest tested concentration of 10 µL. The inhibition subsequently increased to 59.5%, 69.29%, and 89.5%, respectively, at 20 µL. *N. sativa* oil, camphor, and their combined formulation all demonstrated inhibitory values of 63.77%, 73.13%, and 89.77% at 30 µL. These results indicate that all three samples exhibited anti-inflammatory potential, although camphor showed significantly more inhibiting properties than *N. sativa oil* itself. Among all the investigated samples, the combined formulation exhibited the greatest inhibition, suggesting a potential additive or synergistic effect between camphor and the bioactive components of *N. sativa* oil.

Up to 40 µL, at which maximum inhibition was achieved, a distinct dose-dependent pattern was observed. However, at 50 µL, there was no significant increase, suggesting that the inhibitory response might have stopped. Overall, the formulation exhibited greater anti-inflammatory activity than the individual ingredients, suggesting that camphor enrichment may enhance biological performance of *N. sativa* oil.

3.5.3 Antibacterial Activity

The antibacterial activity of *N. sativa* oil, camphor, and their formulation, evaluated using disc diffusion and serial dilution methods, is summarized in Fig 7 and 8. Fig 7 shows the antibacterial activity of *N. sativa* oil, camphor, and their combined formulation on agar plates, where larger clear zones indicate stronger inhibition of bacterial growth with increasing concentrations, whereas Fig 8 presents the measured diameters of inhibition zones in graphical form, showing a

concentration-dependent increase in antibacterial activity, with the combined formulation producing greater inhibition than the individual samples.

The antibacterial activity of *N. sativa* oil, camphor, and their combined formulation were evaluated using the agar disc diffusion method and serial dilution method. In the disc diffusion assay, antibacterial activity was determined by measuring the diameter of inhibition zones around the sample-loaded discs.

Camphor demonstrated larger inhibition zones than *N. sativa* oil, particularly at the lower tested concentrations, compared to the antibacterial assay, indicating more effective antibacterial activity. Among the examined samples, the mixture of *N. sativa* oil and camphor produced the largest inhibition zones, indicating that the combined preparation was more effective against the tested bacteria than either camphor or *N. sativa* oil used on their own.

The results of the serial dilution assay were consistent with those obtained from the disk diffusion assay. Reduced absorbance at 600 nm indicated lower bacterial growth in the presence of the test samples. Among the three samples, the combined formulation produced the greatest reduction in bacterial growth, followed by camphor and *N. sativa* oil. The enhanced activity of the formulation may be related to the combined action of the bioactive constituents present in Camphor and *N. sativa* oil. Overall, the antibacterial activity followed the order:

Camphor–*N. sativa* oil formulation > Camphor > *N. sativa* oil

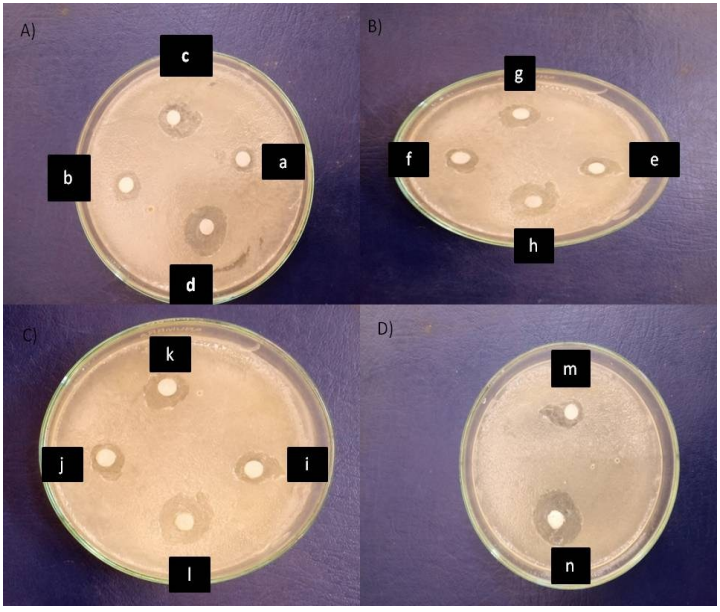


Figure 7. Zone of inhibition produced by different concentrations of *N. sativa* oil, camphor, and their combination against bacterial colonies on agar plates. (A) *N. sativa* oil prepared in n-hexane at 0.1 (a), 0.2 (b), 0.3 (c), and 0.4 mg/mL (d). (B) Camphor prepared in DMSO at 0.1 (e), 0.2 (f), 0.3 (g), and 0.4 mg/mL (h). (C) *N. sativa* oil–camphor formulation prepared in n-hexane at 0.1 (i), 0.2 (j), 0.3 (k), and 0.4 mg/mL (l). (D) Standard antibiotic (m) and untreated control (n)

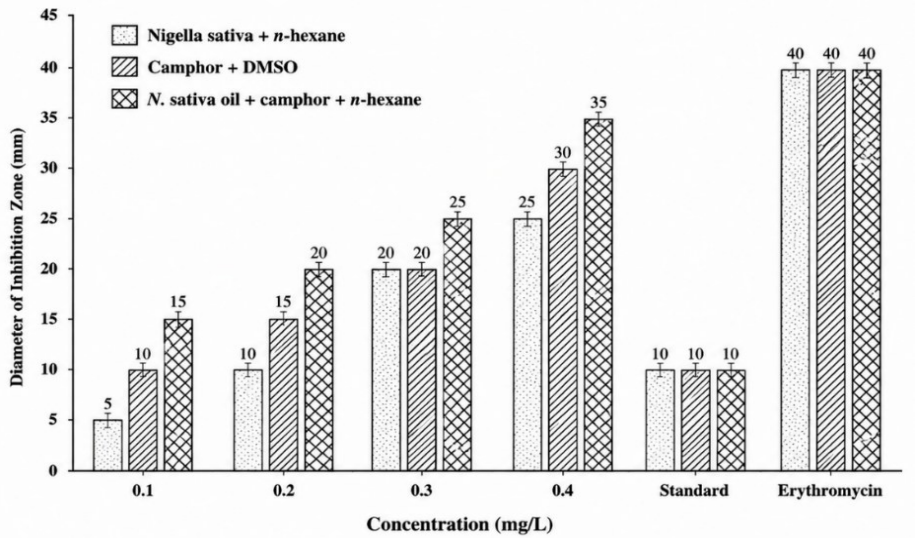


Figure 8. Diameters of the inhibition zones produced by *N. sativa oil*, camphor, and the combined formulation at different concentrations. The results show how antibacterial activity fluctuates with concentration when compared to the standard and erythromycin controls

Standard: Reference control used for comparison. *Erythromycin:* Positive control used as the standard antibiotic. Results are expressed as mean inhibition zone (mm) ± standard deviation (SD) for three replicates (n = 3).

4. DISCUSSION

The results of the research offer important new information about the physicochemical characteristics of *N. sativa oil*. The amount of oil extracted from the seeds was 40.21%, which was comparable to the 37.33% reported by Khoddami *et al.* (2011). The difference between the two numbers could be due to differences in the source of the seed, extraction methods, or analytical factors. *N. sativa* seeds may provide a significant amount of extractable oil with potentially advantageous bioactive components, according to the relatively high yield of the current study.

The ash level of 6.74% that was detected was quite similar to the 6.72% that Khoddami *et al.* (2011) reported. The identical results found in both trials indicate that the ash content of *N. sativa* oil stayed within a similar range despite differences in sample sources and laboratory conditions. The peroxide and free fatty acid (FFA) values in this study were 3.995 ± 0.05 mEq/kg and 0.647 ± 0.001, accordingly. Khoddami *et al.* (2011), on the other hand, recorded a peroxide value of 6.72 ± 0.04 mEq/kg and an FFA value of 6.55 ± 0.01. The lower peroxide value observed in this study might represent an indication of the improved oxidative stability of oil and could also be related to variations in handling, processing, or storage settings.

Saponification and iodine values provide important information regarding fatty acid content and degree of unsaturation of oil. In the current study, *N. sativa* oil has an iodine value of 124 and a saponification value of 189.37. These values indicate fatty acid characteristics that were comparable to those reported previously by Khoddami *et al.* (2011). The results showed strong agreement, indicating that these characteristics of *N. sativa* oil are

relatively stable, which could possibly describe whether it is suitable for utilization in food, medicine, and other industrial settings.

Overall, the credibility of the analytical data is reinforced by substantial agreement between several physicochemical parameters obtained in this study compared to those published in previous work by Khoddami *et al.* (2011). The similarities and discrepancies further indicate that variables including the source of the seed, the extraction process, preservation, and sample handling might affect specific *N. sativa* oil quality parameters. These results might serve as a useful starting point for additional research on the oil's composition, characterization, and possible uses.

The resulting formulation, camphor, and *N. sativa* oil all exhibited distinct volatile compositions, based on the GC-MS results. The monoterpene ketone structure of camphor was in line with the prominent peak observed in its pure form. *N. sativa* oil, in contrast, showed a more complicated profile with a variety of significant chemical compounds, such as p-cymene, thymoquinone, carvacrol, and longifolene. Thymoquinone, carvacrol, longifolene, and other terpenoid molecules have been identified as belonging among the main components of *N. sativa* oil and were attributed to its antioxidant and other biological properties in previous research that showed similar chemical profiles for the oil. The formulation chromatogram maintained the distinctive peaks of both camphor and *N. sativa* oil, indicating that camphor was physically integrated into the oil matrix rather than chemically changed. Since monoterpenes and oxygenated terpenoids are known to contribute to antibacterial, antioxidant, and anti-inflammatory activities, the combination of camphor with thymoquinone, p-cymene, carvacrol, and longifolene could be responsible for the increased biological activity of the combined formulation. Similar reports have described camphor, eucalyptol, borneol, and related terpenoids as major volatile components associated with the biological activity of camphor-containing oils. The appearance of multiple peaks with varying intensities indicated the chemically diverse nature of *N. sativa* oil containing terpenes, quinones and sesquiterpene derivatives. Similar chromatographic profiles of *N. sativa* oil rich in thymoquinone and p-cymene have been reported previously by Nickavar *et al.* (2003). Therefore, the GC–MS findings support the compatibility of both components and indicate that the improved activity of the formulation may be due to the additive contribution of their preserved volatile bioactive constituents (Kiralan *et al.*, 2014; Lee *et al.*, 2022).

FTIR analysis confirmed the physical incorporation of camphor into *N. sativa* oil, as the formulation retained the major functional group bands of both components without the formation of new prominent peaks. The absorption bands around 2922–2853 cm^{-1}, 1744 cm^{-1}, and 1165 cm^{-1} corresponded to aliphatic C–H stretching, ester carbonyl C=O stretching, and C–O stretching of triglycerides, respectively, which are characteristic of *N. sativa* oil and other vegetable oils. The formulation spectrum showed slightly broadening due to the camphor-related carbonyl contribution, which was also observed in the carbonyl region. As a result, the FTIR results show that the components were mostly physically combined, with minimal chemical change between them (Tsai *et al.*, 2023).

In the thermogravimetric Analysis (TGA), following the initial volatilization of camphor, the *N. sativa* oil matrix underwent thermal degradation, exhibiting a stepwise weight-loss pattern. The presence of these separate degradation stages suggests that camphor remained physically compatible with the oil under the conditions used for TGA. The results also provide no clear evidence of a major

chemical interaction between the two components. However, these findings alone cannot establish long-term storage stability or handling safety, as such conclusions would require additional studies, such as accelerated stability or oxidative stability testing, which were not included in the present work.

The DPPH assay showed concentration-dependent antioxidant activity in *N. sativa* oil, camphor, and their formulation. The stronger activity of *N. sativa* oil may be associated with bioactive constituents such as thymoquinone, p-cymene, carvacrol, phenolics, and tocopherols, which are known to contribute to free-radical scavenging potential. The enhanced activity of the camphor–*N. sativa* oil formulation may be due to additive interactions between camphor and the antioxidant constituents of the oil. Recent studies also support the antioxidant potential of black cumin seed oil and its thymoquinone-rich fraction, while camphor-containing essential oil mixtures have also been reported to contribute to improved radical-scavenging activity (Elbouzidi *et al.*, 2024; Chatterjee *et al.*, 2025).

The observed inhibition of protein denaturation by *N. sativa* oil, camphor, and their formulation is indicative of anti-inflammatory potential and may be attributed to the presence of bioactive terpenoid constituents. The higher activity of the formulation suggests an additive effect between camphor and *N. sativa* oil compounds, particularly thymoquinone, p-cymene, carvacrol, and other volatile constituents. Recent studies reported that *N. sativa* and thymoquinone exhibit anti-inflammatory effects through modulation of inflammatory mediators, oxidative stress, and cytokine-related pathways, while camphor-containing essential oils have also been shown to reduce inflammatory cytokines such as TNF-α, IL-6, and IL-1β. Therefore, the enhanced inhibition observed in the formulation supports its potential as a natural anti-inflammatory preparation (Alberts *et al.*, 2024; Zhang *et al.*, 2024).

The antibacterial activity of *N. sativa* oil, camphor, and their formulation may be linked to their volatile bioactive constituents. Compounds such as thymoquinone, p-cymene, carvacrol, and longifolene in *N. sativa* oil are known to disrupt microbial membranes and interfere with bacterial growth, while camphor and related monoterpenes may enhance membrane permeability. The larger inhibition zones observed for the combined formulation suggest an additive effect between camphor and *N. sativa* oil constituents, resulting in stronger inhibition of bacterial growth than that observed with the individual samples. Recent studies also support the antimicrobial potential of *N. sativa* essential oil and its thymoquinone-rich composition against bacterial pathogens (Bhavikatti *et al.*, 2024; Chatterjee *et al.*, 2025).

5. CONCLUSION

This study demonstrates that incorporating camphor into *N. sativa* oil is chemically compatible and physically stable, as indicated by GC–MS and FTIR analyses and supported by TGA, without evidence of major new chemical species relative to the individual components. The mixed formulation exhibited enhanced biological antioxidant, anti-inflammatory, and antibacterial activity as compared with either *N. sativa* oil or camphor, suggesting an eventual additive or synergistic interaction between their bioactive constituents. These results demonstrate the feasibility of the Camphor–*N. sativa* oil combination as a promising natural bioactive formulation for antibacterial, cosmetic, or therapeutic purposes. Although the current evaluation was confined to *in vitro* tests and did not include mechanistic, cytotoxic, and *in vivo* validation; further investigation of these aspects, in addition to stability testing under

various storage conditions, would help in determining the translational potential of the combined formulation.

Declarations

Conflict of Interest Statement

The authors declare that they have no conflict of interest regarding the publication of this research work.

Author Contributions

SK: Investigation, Formal analysis, Methodology, Writing – original draft. **MK:** Investigation, Formal analysis, Writing – original draft. **HA:** Writing – review & editing, Supervision. **JZ:** Writing – review & editing, Supervision. **RB**: Investigation, Formal analysis, Writing – original draft. **TR:** Methodology, Writing – review & editing, Supervision.

Ethics Approval

No ethical approval was required for this study as it did not involve human or animal subjects.

Generative AI Disclosure

The authors confirm that no generative artificial intelligence tools were used in this manuscript.

Availability of Data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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