Experimental and Docking Evidence for Black Seed Oil Nanoemulsion as a Natural Antimicrobial Material

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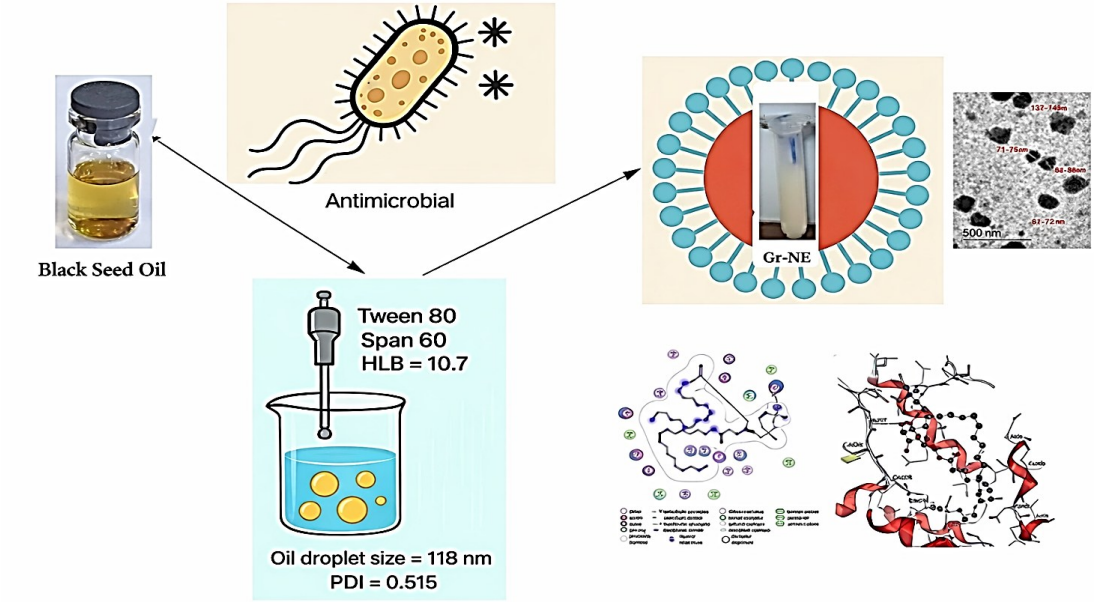
GRAPHICAL ABSTRACT



Experimental and Docking Evidence for Black Seed Oil Nanoemulsion as a Natural Antimicrobial Material

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As more people use chemical preservatives and antibiotics, there is a growing need for safe, natural alternatives. It was posited that black seed oil (*Nigella sativa*) (BSO) and its nanoemulsion (BSO-NE) might exhibit significant antibacterial efficacy against clinically pertinent pathogens. To verify this, BSO-NE was synthesized utilizing the emulsion inversion point (EIP) technique, yielding stable nanoscale oil-in-water droplets, which were validated by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). The antimicrobial activities of BSO and BSO-NE were tested against a group of Gram-positive and Gram-negative bacteria using CFU-reduction assays and agar well diffusion. BSO strongly inhibited the growth of *Staphylococcus epidermidis* (96.6% CFU reduction), while BSO-NE showed varying but still significant activity against the strains that were tested. To investigate the mechanism, molecular docking of thymoquinone and thymohydroquinone with ATPase demonstrated greater binding affinities compared to the reference ligand, corroborating the experimental results. These results show that BSO could be a natural antimicrobial agent and that improving NE formulations could make them work even better. The research highlights the potential of essential-oil-based nanostructures as scalable options for pharmaceutical, biomedical, and food preservation applications.

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Keywords: Nanoemulsion; Antimicrobial activity; Molecular docking; Essential oil; CFU.

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INTRODUCTION

There is growing evidence that essential oils and complex plant extracts may help alleviate some of the issues that arise when microbes become resistant to conventional antibiotics. Numerous studies indicate that essential oils extracted from aromatic plants retain efficacy against multidrug-resistant bacterial strains. One study found that oils derived from thyme, tea tree, bergamot, and lavender were highly effective against both Gram-positive and Gram-negative bacteria, including pathogens that may be resistant to multiple antibiotics (Abdulah *et al.* 2024). Review analyses further contend that the significant chemical complexity of plant extracts—frequently comprising numerous bioactive constituents—necessitates multiple concurrent adaptations by microbes for survival, thereby rendering the emergence of stable resistance considerably less probable compared to single-compound antibiotics. Some essential oils have shown not only the ability to kill bacteria but also the ability to break down biofilms, which is very useful

because biofilms can protect bacteria from regular antibiotics (Khaled *et al.* 2021). In summary, these results indicate that essential oils and plant extracts may serve as a viable, resistance-resistant alternative or adjunct to conventional antimicrobials.

Foodborne illnesses, respiratory tract infections, skin conditions, and systemic complications are all caused by bacterial infections, which continue to be a major global health concern (Gonzalez-Martin 2019; Janikddfghx *et al.* 2020). Particularly, foodborne pathogens continue to pose a major threat to global health and the economy (Amjad *et al.* 2018; Ishaq *et al.* 2021; Ullah *et al.* 2023a,b). Since they have broad-spectrum antimicrobial activity, low toxicity, and a lower chance of negative effects than synthetic preservatives, essential oils made from medicinal plants and spices have drawn more attention in this regard (Noshad *et al.* 2018, 2022; Alizadeh Behbahani *et al.* 2019). These plant-based oils are less likely to cause negative health effects and are safer than synthetic additives (Kamel *et al.* 2024). This affects the size of the droplets, the time it takes for the film to drain, and the resistance to coalescence. The choice of surfactant blend, particularly the hydrophilic-lipophilic balance (HLB), is very important for setting the interfacial tension and adsorption kinetics at the oil-water interface (Chen *et al.* 2025). Recent molecular dynamics studies of binary surfactants at oil-water interfaces have shown that mixed surfactant systems have much lower interfacial formation energy, thicker interfacial films, and better stability under stress than single surfactants alone. Likewise, experimental investigations in Bancroft-type systems (where surfactants are soluble in the continuous phase) demonstrate that elevated surfactant concentration and mixed surfactant formulations result in prolonged film drainage times and inhibited droplet coalescence under hydrodynamic and interfacial stress (Chen *et al.* 2025). Recent advancements in nanotechnology have markedly improved the stability and biological efficacy of bioactive compounds by leveraging the distinctive physicochemical characteristics of nanoparticles. With sizes ranging from 1.0 nm to 100 nm, nanoparticles exhibit special physicochemical characteristics and are used in food, medicine, and cosmetics (Dabhane *et al.* 2021; Ghotekar *et al.* 2021; Alamshany *et al.* 2023). Such particles have a very high surface-area-to-volume ratio, surface chemistry that can be changed, and unique interfacial and quantum effects that bulk materials do not have. These traits allow nano-systems to keep bioactive molecules from breaking down, making them more soluble and easier to spread, and letting them be released in a controlled and long-lasting way. Also, the smaller particle size makes it easier for cells to take them in and interact with biological membranes, which improves biological performance. Many examples of nano-enabled stabilization and delivery effects can be found in food, pharmaceuticals, and cosmetics. In these cases, engineered nanostructures have been shown to work better than traditional formulations (Ashoub *et al.* 2023; Tazimifar *et al.* 2025; Razavi *et al.* 2021; Salavati-Niasari *et al.* 2025).

Nanoemulsions are colloidal dispersions with enhanced bioavailability of active ingredients, high stability, and optical clarity (Mcfarland 1907; Lovelyn *et al.* 2011; Fryd and Mason 2012; Gupta *et al.* 2016). Pharmaceuticals, nutraceuticals, and functional foods have all benefited from their use (Sonneville-Aubrun *et al.* 2004; Rao and McClements 2011; Badruddoza *et al.* 2016). Examining the primary components of natural oil between ligands can provide valuable information about their combined effects and assist in identifying ligands that contribute to the synergy for oil components that have been observed. This information is essential for creating innovative treatment plans and optimizing combination therapies (Zhao *et al.* 2023).

Furthermore, the development of targeted antimicrobial agents depends on investigating the molecular mechanisms underlying the activity of essential oils. Molecular

docking is a powerful tool for essential oil research, offering quick insight into how major phytochemicals interact with microbial proteins. Studies show that 1,8-cineole from *Eucalyptus* oil binds effectively to fungal enzymes such as RS, RibD, and DBPS, supporting its antifungal activity, while carvacrol and thymol from *Thymus vulgaris* oil exhibit strong docking affinities toward *Fusarium oxysporum* virulence proteins. These results highlight docking as an efficient method to predict active compounds and guide experimental validation (Dev Sharma *et al.* 2023; Omar *et al.* 2021). Molecular docking studies offer insightful information about the interactions between bioactive compounds and microbial proteins (Khattab *et al.* 2021; Noshad *et al.* 2022; Agarwal and Smith 2023).

In comparison to past reports of *Nigella sativa* oil's antimicrobial activity, this study presents a number of new features. The emulsion inversion point (EIP) method, a straightforward, low-energy, and repeatable technique that produces nanoscale droplets with improved stability and dispersibility, was first used to create the nanoemulsion (NE) of black seed oil (*Nigella sativa*) (BSO). Second, this study used colony-forming units (CFU) reduction and agar well diffusion assays to systematically compare the antibacterial activity of bulk BSO and its nanoemulsion against both Gram-positive and Gram-negative strains. Most significantly, the study combined computational and experimental methods by using molecular docking simulations to evaluate the binding interactions of the two main bioactive compounds, thymoquinone and thymohydroquinone, with bacterial ATPase (PDB ID: 6zhh). In addition to supporting the microbiological tests, the docking data offer mechanistic insights into BSO's possible antibacterial activity. This work contributes to the understanding of BSO-derived nanostructures as promising natural antimicrobial candidates for pharmaceutical, biomedical, and food preservation applications by bridging the gap between nanoformulation, experimental validation, and *in silico* molecular targeting.

EXPERIMENTAL

Materials and Methods

Materials

Black seed oil (*Nigella sativa*) (BSO) was obtained from the National Research Centre, Egypt. As surfactants and co-surfactants, polysorbate 80 (Tween 80) and sorbitan monostearate (Span 60) (Sigma-Aldrich, USA) were employed. Every chemical used was of analytical grade and didn't require any additional purification. *Shigella flexneri* (ATCC 12022), *Micrococcus luteus* (ATCC 10240), *Bacillus subtilis* (ATCC 6633), and *Staphylococcus epidermidis* (ATCC 12228) were among the test bacterial strains. The reference antibiotic was gentamicin (10 µg/disc, Bioanalyse).

Preparation of Nanoemulsion

The emulsion inversion point (EIP) method was used to formulate BSO nanoemulsion (BSO-NE). Tween 80 and Span 60 (5:1, v/v) were combined with the oil phase (BSO), and then distilled water was added gradually while being constantly stirred until a transparent dispersion was achieved.

Stability Evaluation

Thermodynamic stability tests were performed on the formulations, which included centrifugation (2800 g, 30 min), freeze-thaw cycles (-20 °C/25 °C, 3 cycles), and heating-

cooling cycles (4 °C/45 °C, 3 cycles). Samples exhibiting no coalescence, turbidity, or phase separation were chosen for characterization because they were deemed stable (Deshmukh *et al.* 2017).

Particle Size and Morphology

Following mild sonication, dynamic light scattering (Nano-ZS, Malvern Instruments, UK) was used to measure the mean droplet size and polydispersity index (PDI). Using carbon-coated copper grids, morphological analysis was performed using transmission electron microscopy (TEM, JEOL JEM-2100).

Antimicrobial

Materials

The following were used for the experiments: 100 mL of clean conical flasks, a set of samples, nutrient broth medium (NB), and nutrient agar media (NA). As a general culture medium, nutritional agar (NA) was used for growing and isolating non-picky microbes and for setting up long-term cultures. Yeast extract (2.0 g), peptone (5.0 g), beef extract (1.0 g), sodium chloride (5.0 g), and agar (15.0 g) were its constituent parts. The acidity level was 7.4 ± 0.2. The composition of the nutrient broth medium (NB) in grams per liter was as follows: 2.0 g of yeast extract, 5.0 g of peptone, 1.0 g of meat extract, and 5.0 g of NaCl. The pH was 7.4 ± 0.2. Table 1 shows the list of the tested materials.

The following microorganisms were utilized in this *in vitro* antibacterial investigation: The Gram-negative bacterium *Shigella flexneri* (ATCC 12022) was used. Additionally, the Gram-positive bacteria *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), and *Micrococcus luteus* (ATCC 10240) were also used.

Table 1. The Tested Materials for Antimicrobial Assay

Sample Code	Sample Name
Sample (BSO-NE)	Black seed oil Nano emulsion
Sample (BSO)	Black seed oil
Sample (BSO_{50})	Black seed oil mixed with DMSO [1:1, (v/v)]

Biological Methods

The nutritional broth served as the setting for the qualitative assessments. The microorganisms that were utilized in this study were Gram-positive (*Staphylococcus epidermidis*, *Micrococcus luteus*, and *Bacillus subtilis*) and Gram-negative (*Shigella flexneri*) bacteria that were cultured overnight in nutrient broth and then incubated at 37 °C (Hafez *et al.* 2023). Approximately 0.5 McFarland standard (1.5×10^1 CFU /mL) was used to prepare and alter the inoculum size of this pathogenic strain (1.5×10^8 CFU /mL) (Hafez *et al.* 2023). Each microorganism strain's 25.0 µL inoculum size was separately inoculated into each plate containing 20.0 mL of the sterile nutrient agar medium (NA).

The samples were divided into 2 main groups. In the first group, the sample was applied after the media had cooled and solidified on a 0.6 cm well of the inoculated agar plates, which were prepared previously by using a 0.6 cm cork borer, applying the Well Diffusion Method. The tested sample was added to each well individually using 75.0 µL in this technique (El-Masry *et al.* 2023). Zones of inhibition (ZI) were determined in millimeters after incubating the inoculation plates at 37 ºC for 24 h and placing them in the

refrigerator for an additional hour to allow the samples to diffuse further (Tohamy and El-Masry 2024).

To find out how effective these tested strains were as antimicrobials, the second group of bacteria was cultured in small conical flasks with 20.0 mL of nutrient broth medium and 100.0 µL of bacterial suspensions (0.5 McFarland standard, 1.5 × 108 CFU/mL). The shake flask method was then used to calculate the percentage reduction of colony-forming units (CFU). Each inoculation flask had its discs of tested material added to it individually, and then they were incubated at 37 ºC for 24 h with shaking at 120.0 rpm (Hamoda *et al.* 2022). A sample-culture mixture and controls for each strain were included in a serial dilution from each flask (10-4). One way to find out how much of an impact the treatment had on microbes was to compare the percentage drop in colony-forming units (CFU) between the treated and untreated samples using the following formula (Hamoda *et al.* 2022),

$$\text{Relative reduction (\%)} = [A - (B/A)] \times 100 \quad (1)$$

where A is determined in CFU/mL using the untreated control sample that contains pathogenic strains only without any treatment; and B is determined in CFU/mL using the treated sample tested.

Molecular Docking Study

Gaussian 09 software was used to optimize the 3D structures of thymoquinone and thymohydroquinone, which were then converted into PDB format (Bashar *et al.* 2024; El-Tabakh *et al.* 2025). The RCSB Protein Data Bank (http://www.rcsb.org) provided the ATPase enzyme's crystal structure (PDB ID: 6zhh). Using AutoDock Vina, docking simulations were run to assess ligand–protein interactions. The co-crystallized ligand was re-docked in order to verify the docking reliability. Results were compared with experimental antimicrobial data after binding affinities and interaction patterns were examined (Ahmed *et al.* 2023; Shehata *et al.* 2023).

RESULTS AND DISCUSSION

A thorough evaluation of the physicochemical and functional properties of the formulated black seed oil nanoemulsion (BSO-NE) was conducted to ascertain the correlation among formulation parameters, droplet characteristics, and antimicrobial efficacy. The optimized oil-in-water emulsification process resulted in stable nanosized droplets characterized by a narrow distribution and elevated colloidal stability, demonstrating the effective selection of surfactants and processing conditions. These results show how nanoscale dispersion can greatly increase the solubility, bioavailability, and biological activity of plant-based oils like BSO. The following sections provide in-depth examinations of droplet size, zeta potential, morphology, and chemical stability, accompanied by the relevant antimicrobial and molecular docking findings, to elucidate the mechanisms responsible for the augmented biological activity of BSO-NE.

The chemical profiling of black seed oil (BSO) by GC–MS identified thymoquinone, thymohydroquinone, p-cymene, and carvacrol as major bioactive compounds responsible for its antimicrobial activity. These phenolic and quinone-based constituents form the basis of the enhanced effects observed in the nanoemulsion (BSO-NE). Evaluation of the BSO-NE showed that formulation factors—particularly droplet size, zeta potential,

polydispersity index, and colloidal stability—strongly influence droplet formation, stability, and antibacterial performance. These findings align with established nanoemulsion principles, clarifying the mechanisms underlying its antimicrobial effectiveness.

Composition of *Nigella sativa* Essential Oils and Principal Active Components

C–MS analysis of BSO revealed key antimicrobial compounds—thymoquinone, thymohydroquinone, p-cymene, carvacrol, and thymol—whose membrane-disruptive and metabolic-interfering actions explain the strong antibacterial activity of BSO and BSO-NE, as reflected in the marked CFU reduction and inhibition zones (Khan 1999; Ahmad *et al.* 2013; Farag *et al.* 2020). The most important bioactive of these oils is thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone), which has a wide range of pharmacological effects, such as antibacterial, anti-inflammatory, antioxidant, and anticancer qualities. The phenolic monoterpenes carvacrol (5-isopropyl-2-methylphenol) and thymol (5-methyl-2-isopropylphenol) are also important components, as is *p*-cymene (1-methyl-4-isopropyl-benzene), morphinan-6-ol, 4,5-epoxy-N-methyl-2-[(4-trifluoromethyl) phenoxy], methyl 10-*trans*,12-*cis*-octadecadienoate, and *trans*-farnesol, which is often the most prevalent monoterpene hydrocarbon (Fig. 1). Strong antimicrobial and radical-scavenging properties make carvacrol and thymol stand out, and they frequently work in concert with thymoquinone to increase the essential oil's therapeutic potential (Hannan *et al.* 2021). Other substances that enhance the phytochemical profile and add to bioactivity include thymohydroquinone, dithymoquinone, and trace terpenes (such as 4-terpineol and α-thujene). The well-established antimicrobial effectiveness of black seed essential oil, particularly against Gram-positive and Gram-negative bacterial strains, is based on the combined action of these volatile constituents (Forouzanfar *et al.* 2014).

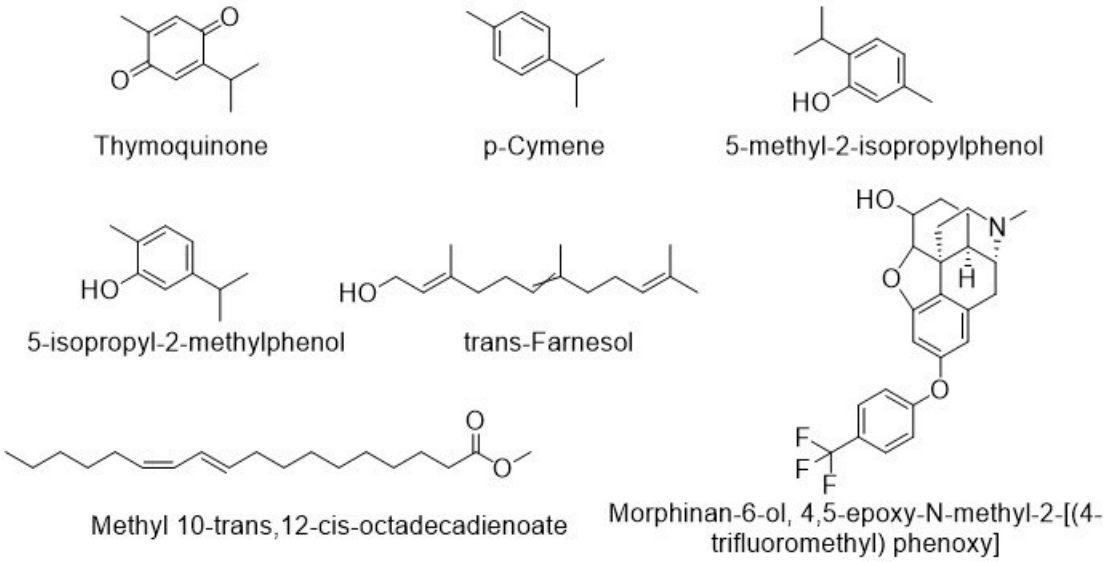

Fig. 1. Major chemical constituents of BSO

Nanoparticles Synthesis and Characterization

BSO Nanoemulsion preparation

The black seed oil nanoemulsion (BSO-NE) was created using a standard oil-in-water (O/W) emulsification method. This made a stable colloidal dispersion of nanosized

oil droplets (about 20 to 200 nm) in a water-based continuous phase. To get the right hydrophilic–lipophilic balance (HLB) for nanoemulsion stability, two nonionic surfactants, Tween 80 (polyoxyethylene sorbitan monooleate) and Span 60 (sorbitan monostearate), were used at a total concentration of 5% (w/v). To make the aqueous phase, the surfactants were mixed with deionized water at room temperature (25 ± 2 °C) for 15 min while being stirred by a magnet at 600 rpm. This made sure that they were completely dissolved and evenly spread out.

Fig. 2. Preparation of BSO-NE

After that, black seed oil was added drop by drop to the surfactant solution while a mechanical homogenizer (IKA T25 Digital Ultra-Turrax, Germany) kept the solution stirring at 10,000 rpm for 15 min. This step made it easier to make a fine, thermodynamically stable nanoemulsion by reducing the size of the droplets. The BSO-NE that came out of this was then put through ultrasonication at 40 kHz for 5 min to make the droplets even smaller and more uniform. The formulation looked clear and didn't have any phase separation, which meant that the emulsification worked, and the colloidal stability was high, as shown in Fig. 2.

Thermodynamic stability of BSO-NE

The thermodynamic strength of the prepared BSO-NE was tested by putting them through a range of accelerated stress tests to see how stable they were. The formulations were put through centrifugation, heating and cooling cycles, and freeze-thaw transitions according to standard procedures (Osanloo *et al.* 2020). The samples were spun at 5,000 rpm for 30 min during centrifugation to see if they were likely to cream, sediment, or separate into phases. In the heating and cooling test, nanoemulsions were stored at 4 °C and 45 °C for six 24-h cycles, with each cycle lasting 24 h. This was done to mimic how the temperature changes when the nanoemulsions are handled and stored. The freeze-thaw test involved exposing samples to −20 °C and 25 °C three times for 24 h each to see how well they could handle big changes in temperature.

After these tests, all of the chosen formulations stayed physically stable, with no signs of turbidity, coalescence, creaming, or phase separation. This showed that they had great thermodynamic stability and interfacial integrity (Rao and McClements 2012; Sengar *et al.* 2018; Pavoni *et al.* 2020). The optimized surfactant combination (Tween 80 and Span 60) made the oil droplets more stable by lowering the interfacial tension and creating a strong steric barrier around them. As a result, droplet aggregation and Ostwald ripening were kept to a minimum, which kept the nanoemulsion's droplet size and optical clarity consistent during the stress tests.

Characterization of Nanoemulsion

The average droplet size of the formulated BSO-NE was about 118 nm, as shown in Fig. 3a. This means that a nanoscale emulsion system was successfully formed. The relatively small droplet size makes it easier for them to pass through skin channels, which makes it easier for them to spread evenly across biological membranes. Rao and McClements (2011, 2012) reported that nanoemulsions with droplet diameters less than 200 nm usually have homogeneous colloidal properties and better kinetic stability because they are less likely to separate and come together due to gravity. Zeta potential measurements also showed that the BSO-NE droplets had a negative surface charge of −17.9 mV (Fig. 3b). This means that the particles were strongly repelling each other through the electrostatic force. This high surface potential stops droplets from coming together and helps the nanoemulsion stay stable over time. The nanoscale droplet size and high zeta potential values work together to show that the BSO-NE system was uniform, stable, and well-dispersed. This makes it good for use on the skin and in medicine.

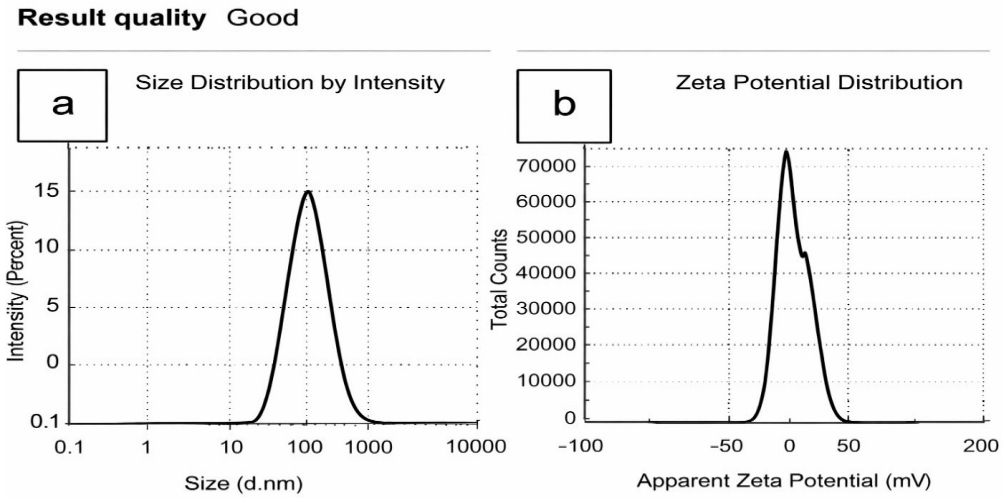

Fig. 3 a) Particle size for BSO-NE, b) Zeta Potential of BSO-NE

Table 2 shows the particle size analysis (PSA) of the prepared BSO-NE. It indicated a polydispersity index (PDI) of 0.515, which means that the size of the droplets was fairly narrow. This value is a little higher than the ideal range for highly monodisperse systems (PDI < 0.3), but it still shows that nanoemulsions made by high-shear homogenization are fairly homogeneous. This kind of uniformity helps the physicochemical behavior, which stays the same, and the diffusion characteristics also stay the same. Moreover, zeta potential analysis (Fig. 3b) indicated that the BSO-NE droplets possessed a negative surface charge of −17.9 mV, thereby validating the existence of electrostatic repulsion among dispersed particles. This negative potential helps to keep colloids stable by stopping droplets from coming together and forming larger droplets while they are being stored. The results of the particle size, PDI, and zeta potential all show that the BSO-NE formulation has good physical stability and even dispersion, which makes it better for topical or pharmaceutical use.

Table 2. Particle Size and Zeta Potential of BSO-NE

Compound	MPS (nm)	PDI	SD	Zeta Potential
BSO-NE	118.30	0.515	±5.94	-17.9

Transmission electron microscopy

Transmission electron microscopy (TEM) was used to look at the shape, size, and spread of the BSO-NE droplets. Figure 4 shows that the TEM micrographs, which showed that the nanoemulsion had spherical nanoparticles that were evenly spread out in the aqueous phase. There were no signs of aggregation, coalescence, or irregular clustering. The diameters of the droplets ranged from 78.2 to 137.1 nm, which was very close to the values found using dynamic light scattering (DLS) measurements. This confirmed that the size analysis was correct. The clear spherical shape and even droplet distribution show that the emulsification process worked well, creating a stable colloidal system with nanoscale droplets of the same shape. The optimized mix of polysorbate 80 and sorbitan monostearate 60, which effectively lowered the interfacial tension between the oil and water phases, is mostly given credit for this morphological uniformity. The surfactant molecules also made a protective film at the interface that provided steric stabilization, which kept droplets from getting too close to each other and fusing. Such stabilization reduces the chance of droplet growth by coalescence or Ostwald ripening, which is why the BSO-NE formulations have such great physical and thermodynamic stability. Overall, the TEM observations give strong visual proof that the mechanisms for emulsification and stabilization worked together to make a stable, long-lasting, nanoscale nanoemulsion system.

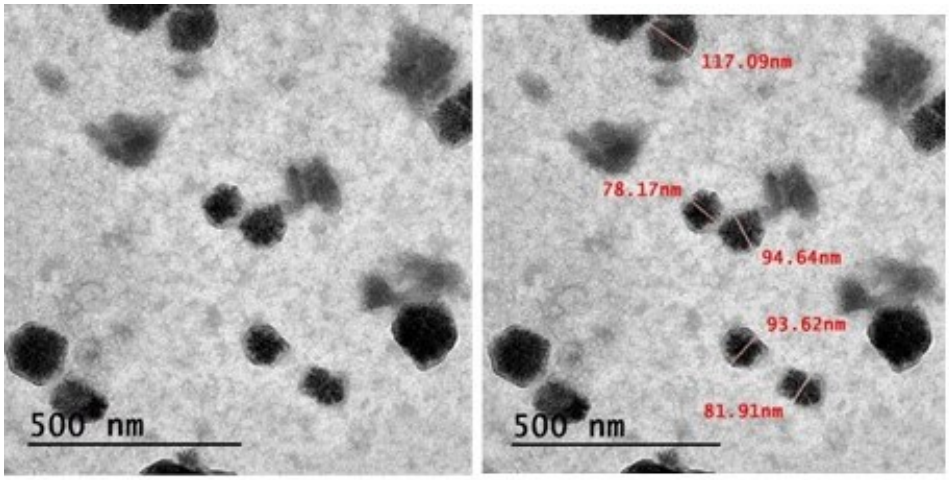

Fig. 4. TEM of BSO-NE

Droplet Stability's Relation to Interfacial and Colloidal Models

Classical colloidal models that describe droplet dynamics in dispersed systems can be used to further interpret the long-term stability of the formulated BSO-NE. In order to preserve the structural integrity of the nanoemulsion, the Laplace pressure—which is inversely proportional to the droplet radius—is essential. The exceptional physical stability seen in centrifugation and thermal cycling tests is a result of the higher internal pressure of smaller droplets (average diameter ≈118 nm), which prevents deformation and coalescence under mechanical or gravitational stress. Furthermore, by encouraging uniform distribution

throughout the aqueous phase, the Brownian motion-driven continuous random motion of the nanosized droplets prevents creaming and sedimentation. According to the measured DLS data, this kinetic stabilization effect takes over when the droplet size is less than 200 nm. Additionally, the BSO-NE system successfully inhibited the Ostwald ripening phenomenon, which occurs when smaller droplets progressively dissolve and redeposit onto larger ones as a result of variations in Laplace pressure. The presence of the mixed nonionic surfactant system (polysorbate 80 and sorbitan monostearate 60), which decreased interfacial tension and slowed molecular diffusion across droplets, and the low solubility of black seed oil in water, were both responsible for this suppression. All of these processes work together to explain why the stability tests showed no discernible changes in droplet size or turbidity. Thus, the observed stability of BSO-NE confirms the strong colloidal stability of the nanoemulsion system and is in good agreement with theoretical predictions based on Laplace pressure equilibrium, Brownian diffusion stability, and Ostwald ripening inhibition.

Antimicrobial Activity

In view of the growing global challenge of antibiotic resistance, identifying natural, safe, and effective antimicrobial alternatives has become increasingly essential. The present study highlights the potent antibacterial activity of BSO and its nanoemulsion (BSO-NE) against a broad range of Gram-positive and Gram-negative bacteria (Tables 3 and 4). Using the shake flask method, it was found that BSO markedly reduced CFU counts of *Bacillus subtilis* (94.87%), *Micrococcus luteus* (92.72%), *Staphylococcus epidermidis* (96.63%), and *Shigella flexneri* (83.41%). This demonstrated its strong and wide-spectrum antimicrobial potential (Table 4). The mechanism of action appears to involve disruption of bacterial metabolic functions and membrane integrity. The bioactive constituents of BSO—particularly thymoquinone, thymohydroquinone, thymol, and carvacrol—interact electrostatically with the negatively charged bacterial membrane, leading to lipid bilayer destabilization, biomolecule leakage, and ATPase inhibition (Hwang *et al.* 2013; Pisoschi *et al.* 2018b). These compounds also interfere with nucleic acid and protein synthesis, resulting in cell death. Such mechanisms are consistent with the bactericidal mode of action, characterized by a ≥99.9% reduction in viable colonies after 24 h incubation (Montgomery and Kroeger 1984). Conversely, bacteriostatic agents inhibit bacterial growth without directly killing the cells (Balouiri *et al.* 2016; Bhargav *et al.* 2016).

Clinically relevant insights were observed for specific pathogens. *Micrococcus luteus* and *Staphylococcus epidermidis*, opportunistic Gram-positive bacteria commonly found on the skin and mucous membranes, showed substantial CFU reductions (92.7% and 96.6%, respectively), indicating BSO's potential for topical or biomedical applications. Infections caused by these bacteria include wound abscesses, cellulitis, and even systemic complications such as endocarditis or bacteremia. Similarly, *Shigella flexneri*, a Gram-negative enteric pathogen associated with foodborne diseases, exhibited notable suppression (83.4%), confirming BSO's efficacy against bacteria with protective lipopolysaccharide barriers. Statistical analysis (one-way ANOVA, $P < 0.05$) verified that all BSO and BSO-NE formulations significantly differed from controls, which showed no inhibition. This indicates that antibacterial activity arose from BSO's bioactive components rather than the surfactants or solvents used. Although bulk BSO demonstrated slightly higher inhibition zones (*e.g.*, *S. epidermidis*, 19.7 mm *vs*. 15.3 mm for BSO-NE), the nanoemulsion exhibited improved colloidal stability and sustained antimicrobial

release, suggesting that encapsulation moderates diffusion kinetics rather than intrinsic efficacy.

According to previous reports, the antimicrobial properties of BSO primarily arise from its ability to interact electrostatically with bacterial outer membranes through its cationic molecules (Pisoschi *et al.* 2018a). This destabilization increases membrane permeability, resulting in the leakage of intracellular materials and the inhibition of essential metabolic enzymes. The combined effects—membrane rupture, ATPase inhibition, leakage of vital biomolecules, and enzyme inactivation—contribute to BSO's potent antibacterial activity (Pisoschi *et al.* 2018a). Molecular docking results further supported these findings, as thymoquinone and thymohydroquinone displayed strong binding affinities (≈ –4.87 kcal mol^{-1}) to bacterial ATPase catalytic sites, reinforcing their role in disrupting energy metabolism and confirming the bactericidal mechanism.

Collectively, the integration of experimental microbiology and computational docking provides a comprehensive mechanistic understanding of BSO's antibacterial action. The novelty of this work lies in employing a low-energy, eco-friendly nanoemulsion system that enhances BSO's stability, bioavailability, and sustained antimicrobial efficacy. This approach establishes BSO-NE as a promising, biocompatible, and long-lasting natural antimicrobial candidate for potential applications in food packaging, pharmaceutical formulations, and wound-healing materials, offering a viable alternative to synthetic antibiotics.

Table 3. Inhibition Zone Diameter (mm) of Tested Samples against Selected Bacterial Strains (Well Diffusion Method, 24 h at 37 °C)

Test Bacterium	Black Seed Oil (BSO)	Nanoemulsion (BSO-NE)	Vehicle Control (Surfactant + Water)	Positive Control (Gentamicin 10 µg/disc)
Staphylococcus epidermidis (G^+)	19.7 ± 0.6^a	15.3 ± 0.5^b	0.0 ± 0.0^c	22.1 ± 0.4^a
Bacillus subtilis (G^+)	18.2 ± 0.4^a	14.1 ± 0.5^b	0.0 ± 0.0^c	18.9 ± 0.3^a
Micrococcus luteus (G^+)	17.5 ± 0.5^a	13.8 ± 0.6^b	0.0 ± 0.0^c	20.4 ± 0.6^a
Shigella flexneri (G^-)	16.1 ± 0.4^a	12.2 ± 0.5^b	0.0 ± 0.0^c	19.2 ± 0.5^a

(Mean ± SD, n = 3; Different superscript letters within each row indicate significant difference at $P < 0.05$ by one-way ANOVA)

Table 4. Percentage Reduction in Colony-Forming Units of Pathogenic Strains (Shake-Flask Method)

Test Bacterium	BSO (%)	BSO-NE (%)	Vehicle Control (%)	Gentamicin (%)
Staphylococcus epidermidis	96.2 ± 1.4^a	83.7 ± 2.1^b	4.5 ± 0.8^c	99.1 ± 0.6^a
Bacillus subtilis	94.6 ± 1.8^a	81.4 ± 1.9^b	5.2 ± 0.6^c	98.7 ± 0.7^a
Micrococcus luteus	93.9 ± 1.7^a	84.1 ± 1.6^b	3.8 ± 0.9^c	98.3 ± 0.9^a
Shigella flexneri	85.5 ± 2.3^a	74.6 ± 2.5^b	2.1 ± 0.5^c	97.8 ± 0.8^a

(Mean ± SD, n = 3; Different superscript letters indicate significant difference at $P < 0.05$)

Multiphase Nanomaterials, Hybrid Systems, and Their Antimicrobial Mechanisms

Recent progress in antimicrobial material design has increasingly focused on multiphase nanostructures, including nanocomposites, core–shell hybrids, and nano-bio hybrid systems, because these architectures combine the advantages of different phases to produce synergistic antimicrobial effects. Several studies cited in the manuscript demonstrate such strategies. For example, plasma-coated $CaSiO_3$/CuO nanocomposite coatings on fabrics exhibit enhanced antibacterial performance through combined metallic ion release and surface-reactive oxygen species generation (Hamoda *et al.* 2022; Safardoust-Hojaghan *et al.* 2021). Similarly, fluffy amphiphilic graphene oxide hybrids incorporated into polymeric hydrogels improve both thermal stability and antibacterial action by combining the high surface area of graphene derivatives with the diffusional properties of polymer matrices (Tohamy and El-Masry 2024; Hassanpour *et al.* 2019). Another example is the *Pyrus communis* extract–nanoparticle hybrid bioinsecticidal system, where plant-derived phytochemicals and nanostructured carriers act cooperatively to disrupt membrane integrity (Shehata *et al.* 2023; Rashki *et al.* 2022). Moreover, sono-synthesized triazole–selenium nanocomposites demonstrated improved anticancer and antimicrobial activity due to the combined biological functionality of the organic moiety and the redox-active selenium core (Khattab *et al.* 2021; Karkeh-Abadi *et al.* 2022).

These multiphase designs operate through multimodal antimicrobial mechanisms, including:

- Cell membrane rupture and increased permeability due to nanoscale surface interactions.
- Synergistic reactive oxygen species (ROS) generation from metal oxide phases.
- Enzyme inhibition and intracellular metabolic disruption caused by organic/plant-derived ligands.
- Sustained release of active components from polymeric or inorganic carriers.

Such mechanisms create a broader antimicrobial spectrum and often outperform single-phase materials.

Molecular Docking

Molecular docking analysis was performed to clarify the possible interactions between the primary bioactive components of BSO—thymoquinone and thymohydroquinone, and the ATPase enzyme active site (PDB ID: 6ZHH). The docking results showed that both compounds had stronger binding affinities (−4.872 and −4.869 kcal/mol, respectively) than the reference ligand that was co-crystallized with them (−4.519 kcal/mol) (Table 4). These negative binding energy values suggest that the interactions happen on their own and are energetically favorable. This means that both thymoquinone and thymohydroquinone can bind well to the enzyme's catalytic pocket. The tested compounds exhibited stronger interaction energies compared to the control ligand, suggesting that these phytochemicals may more effectively inhibit bacterial ATPase activity, thereby contributing to the observed antimicrobial effects *in vitro*. The trend in binding energy was Thymohydroquinone > Thymoquinone > Co-crystallized ligand (PCW), which means that thymohydroquinone has a slightly stronger binding affinity for the target enzyme. This correlates well with the experimental data showing a decrease in colony-forming units (CFUs), which shows that in silico predictions and *in vitro* antimicrobial tests are very accurate.

This kind of consistency between the theoretical and experimental results makes the docking analysis supports its reliability and utility for making predictions. The molecular interactions observed—specifically hydrogen bonding, hydrophobic contacts, and π–π stacking—are likely crucial for stabilizing the ligand–enzyme complexes (Table 5 and Fig. 5). In general, these results show that thymoquinone and thymohydroquinone have strong, specific, and biologically relevant interactions with bacterial enzymes, which explains how they work as antimicrobials.

Table 5. Docking Interaction Data Calculations of Co-Crystallized Control Ligand, and Thymohydroquinone, and Thymoquinone in the Enzyme Binding Pocket of the ATPase Enzyme (PDB ID: 6zhh) Active Site Receptor

Compound	Ligand	Amino Acids	Interaction	Affinity Bond Length (in Å from main residue)	Affinity Bond Strength (kcal/mol)	Energy Score (S) (kcal/mol)
PCW (Control ligand)	C123	ASP45	H-donor	3.3	-1.3	-4.5189
	C131	ASP45	H-donor	3.26	-0.9	
	N126	ASP45	Ionic	3.85	-0.8	
Thymohydroquinone	O9	MET59	H-donor	3.19	-2.7	-4.869
Thymoquinone	O14	LYS228	H-acceptor	3.14	-1.7	-4.872

The molecular docking simulations provided mechanistic data that supported the experimentally reported antibacterial activity of black seed oil and its nanoemulsion. The two primary bioactive chemicals discovered by GC-MS, thymoquinone and thymohydroquinone, displayed high affinity for the bacterial ATPase enzyme (PDB ID: 6ZHH). Their binding energies were −4.872 and −4.869 kcal·mol^{-1}, respectively, which were greater than the co-crystallized reference ligand (−4.519 kcal·mol^{-1}).

Both compounds formed hydrogen bonds and hydrophobic contacts with key catalytic residues (such as ASP45, LYS228, and MET59), which are required for ATP hydrolysis and energy transduction in bacterial cells. This computational data is consistent with the actual results reported in Tables 3 and 4, which reveal that *Staphylococcus epidermidis* and *Bacillus subtilis*—organisms with high ATPase activity—had the most substantial drop in CFU and the greatest inhibitory zones. The inhibitory mechanism is most likely related to disruption of energy metabolism, as ATPase inhibition can reduce proton motive force, impede nutrient transport, and eventually lead to cell death. The nanoemulsion's antibacterial activity is slightly lower than that of bulk oil. This could be because the active quinones are slowly released from nanosized droplets, allowing for prolonged yet modest biological exposure. This study proposes a cohesive mechanistic hypothesis by combining *in silico* and *in vitro* findings: the components of BSO exhibit antimicrobial activities primarily through ATPase inhibition and membrane instability, as predicted by molecular docking and demonstrated experimentally. This integrative interpretation links molecular-level interactions to bigger biological impacts, making this work more novel and scientifically sound.

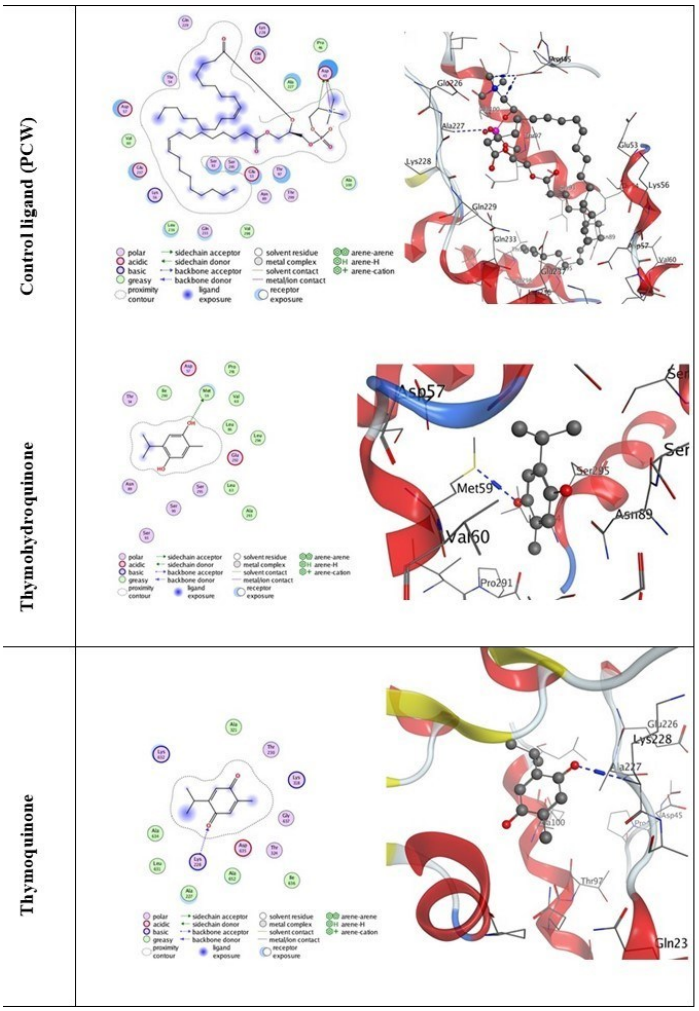

Fig. 5. 2D and 3D molecular docking simulation studies of the co-crystallized control ligand (PCW), Thymohydroquinone, and Thymoquinone in the enzyme binding pocket of the ATPase enzyme (PDB ID: 6zhh) active site receptor

Integration of Findings with the Identified Research Problem and Research Gap

This study directly addressed the need for stable, mechanistically supported natural antimicrobials that can replace synthetic preservatives and conventional antibiotics. Earlier work on *Nigella sativa* oil showed promising activity but suffered from unstable emulsions, limited mechanistic insight, and a lack of integrated nanoformulation and bioactivity analysis. Here, these gaps were closed by linking nanoemulsion formulation, physicochemical stability, antimicrobial performance, and molecular docking in a unified approach. The optimized BSO-NE demonstrated consistent nanoscale droplets, negative zeta potential, and strong resistance to phase separation, confirming that the EIP method produces stable dispersions capable of sustained antimicrobial release. Comparing BSO and BSO-NE also clarified that nano-dispersion enhances bioavailability and antimicrobial action, as reflected in the strong CFU reduction and inhibition zones, particularly against *S. epidermidis* and *B. subtilis*.

Molecular docking further revealed that thymoquinone and thymohydroquinone strongly bind bacterial ATPase catalytic residues, providing a mechanistic explanation that aligns with the observed biological effects. Together, these results fill the major formulation

and mechanistic gaps in previous studies and establish BSO-NE as a stable, effective, and scientifically validated natural antimicrobial system with potential applications in pharmaceutical, biomedical, and food preservation fields.

BSO-NE's Comparative Analysis with Current Essential Oil Nanoemulsion Research

Comparing the physicochemical characteristics and antimicrobial effectiveness of the produced BSO and BSO-NE with comparable nanoemulsion systems documented in recent literature is crucial to contextualizing our findings. According to studies conducted between 2021 and 2025, zeta potential, droplet size, and polydispersity index (PDI) are important factors that affect the stability of nanoemulsions and their biological performance. Furthermore, CFU reduction values, inhibition zones, and concentration requirements offer standards for assessing antimicrobial potency in various formulations. Other recent studies have reported varying results depending on formulation composition, stabilizers, and surfactants, despite our work showing strong antibacterial activity of pure BSO and measurable but lower activity of BSO-NE. Table 6 presents a comparative summary of important metrics that show our formulation's strengths as well as areas that need improvement to meet new standards in essential oil nanoemulsion research.

Table 6. Literature Comparison with this Study

Parameter	**This Study BSO / BSO-NE**	**Mosa *et al.* (2022-2023) –** *Nigella sativa* O/W nanoemulsions *(P. verrucosum)* (Mosa *et al.* 2023)	**Hasanin *et al.* (2025) –** Chitosan-loaded BSO nanoemulsions, (Hasanin *et al.* 2025)
Droplet size	~118 nm (NE, from TEM / DLS)	**168.6 nm to 345.3 nm**, depending on surfactant polysorbate 20 (Tween-20), or polysorbate 80 (Tween-80), and sonication time	"Stable nanoemulsions … with homogeneous particle size distributions in the nanoscale range" — exact value ~200-300 nm (BSO NEO chitosan)
Concentration / Used % for efficacy tests	100% used in CFU reduction and other assays (for both BSO & BSO-NE)	100% concentration of nanoemulsions required to achieve complete inhibition of fungal growth (*P. verrucosum*)	Tested with 1%-3% chitosan load in nanoemulsion; inhibition zones in assays (exactly comparable concentrations)
% Inhibition / Kill Time / Bioactivity	BSO: up to ~96.63% CFU reduction (*e.g.*, *S. epidermidis*); BSO-NE: lower reductions across some strains; molecular docking supports strong ligand-enzyme binding	Complete inhibition of *P. verrucosum* at 100% concentration; also, beneficial effects on seed germination and plant growth parameters	Inhibition zones *vs.* resistant fungi (17-23 mm) with BSO NEO chitosan, outperforming some synthetic treatments; shows strong antifungal bioactivity

CONCLUSIONS

1. This study showed that Black Seed (*Nigella sativa*) Oil has strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Micrococcus luteus* were all shown to be significantly inhibited.
2. Mechanistic evidence for the observed antimicrobial activity was provided by complementary molecular docking studies, which verified that the main bioactive compounds, thymohydroquinone and thymoquinone, have a higher binding affinity to the ATPase enzyme than the co-crystallized ligand.
3. This integrated experimental–computational approach demonstrates BSO (*Nigella sativa*) as a distinct and potent natural alternative to synthetic antimicrobials, in contrast to many earlier studies that only concentrated on conventional assays. All of the results point to BSO as a viable option for use in pharmaceutical formulations, biomedical materials, and food preservation. To fully realize its therapeutic and commercial potential, future research should focus on *in vivo* validation, synergistic formulations, and industrial-scale applications.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethics declaration

Ethics declaration: not applicable.

Authors' Contributions

J.G.E.: Conceptualization, writing, review, and editing. **M.A.A.**: Collect data, write review draft. **R.M.A.:** writing—original draft, formal analysis, and investigation. **N.Q.A.**: formal analysis and investigation. **A.A.E.:** Conceptualization, writing—original draft, and editing the final form.

REFERENCES CITED

Abdulah, Y., Al-Mahdi, Alabed, Ali A. Alabed, Mohammed Faez Baobaid. (2024). "Antibacterial activity of herbal essential oils against gram-positive and gram-negative bacteria with a potential for multidrug resistance," *Journal of Angiotherapy* 8(2), article 9517.

Agarwal, R., and Smith, J. C. (2023). "Speed *vs.* accuracy: Effect on ligand pose accuracy of varying box size and exhaustiveness in autodock vina," *Molecular Informatics* 42(2), article e2200188. https://doi.org/10.1002/MINF.202200188

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., Damanhouri, Z. A., and Anwar, F. (2013). "A review on therapeutic potential of *Nigella sativa*: A miracle herb," *Asian Pacific Journal of Tropical Biomedicine* 3(5), 337-352. https://doi.org/10.1016/S2221-1691(13)60075-1

Ahmed, N., El-Tabakh, M. A. M., Mohamed, H. F., Wu, X., Xu, C., and Huang, L. (2023). "Molecular docking and antibacterial activity of *Sargassum fusiforme* extracts against major coral pathogens," *World Journal of Microbiology and Biotechnology* 39(11), 1-16. https://doi.org/10.1007/s11274-023-03752-8

Alamshany, Z. M., Khattab, R. R., Hassan, N. A., El-Sayed, A. A., Tantawy, M. A., Mostafa, A., Hassan, A. A., and Eg, A. A. H. (2023). "Synthesis and molecular docking study of novel pyrimidine derivatives against COVID-19," *Molecules* 28(2), article 739. https://doi.org/10.3390/molecules28020739

Alizadeh Behbahani, B., Noshad, M., and Falah, F. (2019). "Cumin essential oil: Phytochemical analysis, antimicrobial activity and investigation of its mechanism of action through scanning electron microscopy," *Microbial Pathogenesis* 136, article 103716. https://doi.org/10.1016/j.micpath.2019.103716

Amjad, N., Rehman, M., Khalid, M., and Amjad, I. (2018). "Construction of the infectious molecule of beta satellite associated with ageratum yellow vein disease of ageratum conyzoides," *Bulletin of Biological and Allied Sciences Research* 2018(1), article 13. https://doi.org/10.54112/bbasr.v2018i1.13

Ashoub, M. H., Amiri, M., Razavi, R., Dawi, E. A., Farsinejad, A., Divsalar, F., and Salavati-Niasari, M. (2023). "Induction of ferroptosis cell death in acute promyelocytic leukemia cell lines (NB4 and HL-60) using hydrothermally synthesized ZnO NPs in the presence of black cardamom extract," *Results in Engineering* 20, article 101479. https://doi.org/10.1016/j.rineng.2023.101479

Badruddoza, A. Z. M., Godfrin, P. D., Myerson, A. S., Trout, B. L., and Doyle, P. S. (2016). "Core–shell composite hydrogels for controlled nanocrystal formation and release of hydrophobic active pharmaceutical ingredients," *Advanced Healthcare Materials* 5(15), 1960-1968. https://doi.org/10.1002/ADHM.201600266,

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2016). "Methods for *in vitro* evaluating antimicrobial activity: A review," *Journal of Pharmaceutical Analysis* 71–79. https://doi.org/10.1016/J.JPHA.2015.11.005

Bashar, M. A. E., Hamed, A., El-Tabakh, M. A. M., Hashem, A. H., Zaki, A. A., Al-Askar, A. A., Abou-Amra, E. S., El-Beeh, M. E., Mehany, A. B. M., Shaaban, M. *et al.* (2024). "Anticancer, antimicrobial, insecticidal and molecular docking of sarcotrocheliol and cholesterol from the marine soft coral *Sarcophyton trochelio-phorum*," *Scientific Reports* 14(1), 1-16. https://doi.org/10.1038/S41598-024-75446-6

Bhargav, H. S., Shastri, S. D., Poornav, S. P., Darshan, K. M., and Nayak, M. M. (2016). "Measurement of the zone of inhibition of an antibiotic," *Proceedings - 6th International Advanced Computing Conference, IACC 2016*, pp. 409-414. https://doi.org/10.1109/IACC.2016.82

Chen, Y., Bahadori, N., and Dutcher, C. S. (2025). "Surfactant-laden micro-scale droplet coalescence in Bancroft-breaking systems," *Soft Matter* 21(39), 7710-7725. https://doi.org/10.1039/D4SM00644E

Dabhane, H., Ghotekar, S., Tambade, P., Pansambal, S., Murthy, H. C. A., Oza, R., and Medhane, V. (2021). "A review on environmentally benevolent synthesis of CdS nanoparticle and their applications," *Environmental Chemistry and Ecotoxicology* 3, 209-219. https://doi.org/10.1016/J.ENCECO.2021.06.002

Deshmukh, K., Ahamed, M. B., Sadasivuni, K. K., Ponnamma, D., AlMaadeed, M. A. A., Khadheer Pasha, S. K., Deshmukh, R. R., and Chidambaram, K. (2017). "Graphene oxide reinforced poly (4-styrenesulfonic acid)/polyvinyl alcohol blend composites with enhanced dielectric properties for portable and flexible electronics,"

Materials Chemistry and Physics 186, 188-201. https://doi.org/10.1016/j.matchemphys.2016.10.044

Dev Sharma, A., Kaur, I., and Chauhan, A. (2023). "Compositional profiling and molecular docking studies of *Eucalyptus polybrachtea* essential oil against mucormycosis and aspergillosis," *BioTechnologia* 104(3), 233-245. https://doi.org/10.5114/bta.2023.130727

El-Masry, H. M., Atwa, N. A., El-Beih, A. A., Agwa, M. M., Khafagi, I. K., Mansour, S. R., and El-Diwany, A. I. (2023). "Phenazine-producing *Pseudomonas aeruginosa* OQ158909: A promising candidate for biological activity and therapeutic applications," *Egyptian Journal of Chemistry* 66(11), 281-303. https://doi.org/10.21608/EJCHEM.2023.214109.8045

El-Tabakh, M. A. M., Shehata, A. Z. I., Sadek, A. M., Salem, S. S., Elmehdawy, A. A., Nazih, M., Omar, G. M., Elsoud, A. A. A., Abdel-Aziz, A. N. G., El-Abeid, S. E., Abd-Elkhalek, H. F., and Arief, O. M. (2025). "Multifunctional properties of Phlomis aurea extracts: *In-vitro* antioxidant, antimicrobial, anticancer, potent repellency against two mosquito vectors and molecular docking studies," *Applied Biological Chemistry* 68(1), 1-16. https://doi.org/10.1186/s13765-025-00982-2

Farag, S. M., El-Sayed, A. A., and Abdel-Haleem, D. R. (2020). "Larvicidal efficacy of *Nigella sativa* seeds oil culex pipiens and *Musca domestica*," *Journal of the Egyptian Society of Parasitology* 50(1), 215-220.

Forouzanfar, F., Fazly Bazzaz, B. S., and Hosseinzadeh, H. (2014). "Black cumin (*Nigella sativa*) and its constituent (thymoquinone): A review on antimicrobial effects," *Iranian Journal of Basic Medical Sciences* 17(12), article 929.

Fryd, M. M., and Mason, T. G. (2012). "Advanced nanoemulsions," *Annual Review of Physical Chemistry* 63, 493-518. https://doi.org/10.1146/annurev-physchem-032210-103436

Ghotekar, S., Pansambal, S., Bilal, M., Pingale, S. S., and Oza, R. (2021). "Environmentally friendly synthesis of Cr_2O_3 nanoparticles: Characterization, applications and future perspective ─ A review," *Case Studies in Chemical and Environmental Engineering* 3, article 100089. https://doi.org/10.1016/j.cscee.2021.100089

Gonzalez-Martin, C. (2019). "Airborne infectious microorganisms," *Encyclopedia of Microbiology* 52. https://doi.org/10.1016/B978-0-12-809633-8.13002-X

Gupta, A., Eral, H. B., Hatton, T. A., and Doyle, P. S. (2016). "Nanoemulsions: Formation, properties and applications," *Soft Matter* 12(11), 2826-2841. https://doi.org/10.1039/C5SM02958A

Hafez, A. I., Ali, H. M., Sabry, R. M., El-Masry, H. M., and Abd El-Gawad, W. M. (2023). "Generation of novel, hygienic, inhibitive, and cost-effective nanostructured core-shell pigments," *Progress in Organic Coatings* 175, article 107325. https://doi.org/10.1016/J.PORGCOAT.2022.107325

Hamoda, D. M., Kenawy, S. H., Aboalmaged, H. H., Rashed, U. M., Almetwally, Al. A., and El-Masry, H. M. (2022). "Plasma technique application for coating non-woven fabric by ($CaSiO_3$/CuO) nano particles for biomedical sector," *Egyptian Journal of Chemistry* 65(8), 773-778. https://doi.org/10.21608/EJCHEM.2022.128815.5705

Hannan, M. A., Rahman, M. A., Sohag, A. A. M., Uddin, M. J., Dash, R., Sikder, M. H., Rahman, M. S., Timalsina, B., Munni, Y. A., Sarker, P. P. *et al.* (2021). "Black cumin (*Nigella sativa* L.): A comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety," *Nutrients* 13(6), article 1784. https://doi.org/10.3390/NU13061784

Hasanin, M. S., Ayoob, F. A., Hashem, A. H., and Emam, M. (2025). "Synthesis of Chitosan based nanoemulsions and their characterization and antifungal activity toward fungi causing mucormycosis," *Scientific Reports* 15(1), article 20326. https://doi.org/10.1038/S41598-025-06577-7

Hassanpour, M., Salavati-Niasari, M., Hosseini Tafreshi, S. A., Safardoust-Hojaghan, H., and Hassanpour, F. (2019). "Synthesis, characterization, and antibacterial activities of Ni/ZnO nanocomposites using bis(salicylaldehyde) complex precursor," *Journal of Alloys and Compounds* 788, 383-390. https://doi.org/10.1016/j.jallcom.2019.02.255

Hwang, Y. Y., Ramalingam, K., Bienek, D. R., Lee, V., You, T., and Alvarez, R. (2013). "Antimicrobial activity of nanoemulsion in combination with cetylpyridinium chloride in multidrug-resistant *Acinetobacter baumannii*," *Antimicrobial Agents and Chemotherapy* 57(8), 3568-3575. https://doi.org/10.1128/AAC.02109-12

Ishaq, A. R., Manzoor, M., Hussain, A., Altaf, J., Rehman, S. U., Javed, Z., Afzal, I., Noor, A., and Noor, F. (2021). "Prospect of microbial food borne diseases in Pakistan: A review," *Brazilian Journal of Biology* 81(4), 940-953. https://doi.org/10.1590/1519-6984.232466

Janikddfghx, E., Ceremuga, M., Niemcewicz, M., and Bijak, M. (2020). "Dangerous pathogens as a potential problem for public health," *Medicina (Lithuania)* 56(11), article 591. https://doi.org/10.3390/MEDICINA56110591

Kamel, O. M. H. M., El-halim, M. D. A., Khalil, A. M., Elasasy, M. E. A., and El-sayed, A. A. (2024). "A promising route to control mosquito larvae by metal nanoparticles," *Egyptian Journal of Aquatic Biology and Fisheries* 28(5), 1699-1732.

Karkeh-Abadi, F., Safardoust-Hojaghan, H., Jasim, L. S., Abdulsahib, W. K., Mahdi, M. A., and Salavati-Niasari, M. (2022). "Synthesis and characterization of $Cu_2Zn_{1.75}Mo_3O_{12}$ ceramic nanoparticles with excellent antibacterial property," *Journal of Molecular Liquids* 356, article 119035. https://doi.org/10.1016/j.molliq.2022.119035

Khan, M. A. (1999). "Chemical composition and medicinal properties of *Nigella sativa* Linn.," *Inflammopharmacology* 7(1), 15-35. https://doi.org/10.1007/S10787-999-0023-Y/METRICS

Khaled A. El-Tarabily, Mohamed T. El-Saadony, Mahmoud Alagawany, Muhammad Arif, Gaber E. Batiha, Asmaa F. Khafaga, Hamada A.M. Elwan, Shaaban S. Elnesr, and Mohamed E. Abd El-Hack. (2021). "Using essential oils to overcome bacterial biofilm formation and their antimicrobial resistance," *Saudi Journal of Biological Sciences* 28(9), 5145-5156. https://doi.org/10.1016/j.sjbs.2021.05.033

Khattab, R. R., Swelam, S. A., Khalil, A. M., Abdelhamid, A. E., Soliman, A. M., and El-Sayed, A. A. (2021). "Novel sono-synthesized triazole derivatives conjugated with selenium nanoparticles for cancer treatment," *Egyptian Journal of Chemistry* 64(8), 4675-4688. https://doi.org/10.21608/EJCHEM.2021.81154.4018

Lovelyn, C., Attama, A. A., Lovelyn, C., and Attama, A. A. (2011). "Current state of nanoemulsions in drug delivery," *Journal of Biomaterials and Nanobiotechnology* 2(5), 626-639. https://doi.org/10.4236/JBNB.2011.225075

Mcfarland, J. (1907). "The nephelometer:an instrument for estimating the number of

bacteria in suspensions used for calculating the opsonic index and for vaccines," *Journal of the American Medical Association* XLIX(14), 1176-1178. https://doi.org/10.1001/JAMA.1907.25320140022001F

Montgomery, E. H., and Kroeger, D. C. (1984). "Principles of anti-infective therapy," *Dental Clinics of North America* 28(3), 423-432. https://doi.org/10.1016/B978-0-323-50934-3.00008-2.

Mosa, M. A., Youssef, K., Hamed, S. F., and Hashim, A. F. (2023). "Antifungal activity of eco-safe nanoemulsions based on *Nigella sativa* oil against *Penicillium verrucosum* infecting maize seeds: Biochemical and physiological traits," *Frontiers in Microbiology* 13, article 1108733. https://doi.org/10.3389/FMICB.2022.1108733/FULL

Noshad, M., Alizadeh Behbahani, B., and Nikfarjam, Z. (2022). "Chemical composition, antibacterial activity and antioxidant activity of *Citrus bergamia* essential oil: Molecular docking simulations," *Food Bioscience* 50, article 102123. https://doi.org/10.1016/J.FBIO.2022.102123

Noshad, M., Hojjati, M., and Alizadeh Behbahani, B. (2018). "Black zira essential oil: Chemical compositions and antimicrobial activity against the growth of some pathogenic strain causing infection," *Microbial Pathogenesis* 116, 153-157. https://doi.org/10.1016/j.micpath.2018.01.026

Omar, H. S., Abd El-Rahman, S. N., AlGhannam, S. M., Reyad, N. E.-H. A., and Sedeek, M. S. (2021). "Antifungal evaluation and molecular docking studies of *Olea europaea* leaf extract, *Thymus vulgaris* and *Boswellia carteri* essential oil as prospective fungal inhibitor candidates," *Molecules* 26(20), article 6118. https://doi.org/10.3390/molecules26206118

Osanloo, M., Abdollahi, A., Valizadeh, A., and Abedinpour, N. (2020). "Antibacterial potential of essential oils of *Zataria multiflora* and *Mentha piperita*, micro-and nano-formulated forms," *Iranian Journal of Microbiology* 12(1), 43-51. https://doi.org/10.18502/IJM.V12I1.2517

Pavoni, L., Perinelli, D. R., Bonacucina, G., Cespi, M., and Palmieri, G. F. (2020). "An overview of micro- and nanoemulsions as vehicles for essential oils: formulation, preparation and stability," *Nanomaterials* 10(1), article 135. https://doi.org/10.3390/NANO10010135

Pisoschi, A. M., Pop, A., Cimpeanu, C., Turcuş, V., Predoi, G., and Iordache, F. (2018a). "Nanoencapsulation techniques for compounds and products with antioxidant and antimicrobial activity - A critical view," *European Journal of Medicinal Chemistry* 157, 1326–1345. https://doi.org/10.1016/J.EJMECH.2018.08.076

Pisoschi, A. M., Pop, A., Georgescu, C., Turcuş, V., Olah, N. K., and Mathe, E. (2018b). "An overview of natural antimicrobials role in food," *European Journal of Medicinal Chemistry* 143, 922-935. https://doi.org/10.1016/j.ejmech.2017.11.095

Rao, J., and McClements, D. J. (2011). "Food-grade microemulsions, nanoemulsions and emulsions: Fabrication from sucrose monopalmitate and lemon oil," *Food Hydrocolloids* 25(6), 1413-1423. https://doi.org/10.1016/J.FOODHYD.2011.02.004

Rao, J., and McClements, D. J. (2012). "Food-grade microemulsions and nanoemulsions: Role of oil phase composition on formation and stability," *Food Hydrocolloids* 29(2), 326-334. https://doi.org/10.1016/J.FOODHYD.2012.04.008

Rashki, S., Safardoust-Hojaghan, H., Mirzaei, H., Abdulsahib, W. K., Mahdi, M. A., Salavati-Niasari, M., Khaledi, A., Khorshidi, A., and Mousavi, S. G. A. (2022). "Delivery of LL37 by chitosan nanoparticles for enhanced antibacterial and

antibiofilm efficacy," *Carbohydrate Polymers* 291, article 119634. https://doi.org/10.1016/j.carbpol.2022.119634

Razavi, R., Amiri, M., Alshamsi, H.A., Eslaminejad, T., and Salavati-Niasari, M. (2021). "Green synthesis of Ag nanoparticles in oil-in-water nano-emulsion and evaluation of their antibacterial and cytotoxic properties as well as molecular docking." *Arabian Journal of Chemistry* 14(9), article 103323. https://doi.org/10.1016/j.arabjc.2021.103323

Safardoust-Hojaghan, H., Salavati-Niasari, M., Amiri, O., Rashki, S., and Ashrafi, M. (2021). "Green synthesis, characterization, and antimicrobial activity of carbon quantum dots-decorated ZnO nanoparticles," *Ceramics International* 47(4), 5187-5197. https://doi.org/10.1016/j.ceramint.2020.10.097

Salavati-Niasari, M., Monsef, R., Karami, A., Aljeboree, A. M., Alsultany, F. H., Hamza, H. H. (2025). "Sonochemical preparation and characterization of $ErVO_4$/g-C_3N_4 nano-photocatalysts for removal of water-soluble organic dyes under visible light exposures," *Results in Engineering* 28, article 107764. https://doi.org/10.1016/j.rineng.2025.107764

Sengar, A., Basheer, F., Aziz, A., and Farooqi, I. H. (2018). "Aerobic granulation technology: Laboratory studies to full scale practices," *Journal of Cleaner Production* 197, 616–632. https://doi.org/10.1016/J.JCLEPRO.2018.06.167

Shehata, A. Z. I., Elhawary, E. A., Mokhtar, M. M., Waheeb, H. O., Fares, M., Emam, D. E. M., Bakr, N. A., Sadek, A. M., and El-Tabakh, M. A. M. (2023). "Molecular docking and insecticidal activity of *Pyrus communis* L. extracts against disease vector, *Musca domestica* L. (Diptera: muscidae)," *Egyptian Journal of Basic and Applied Sciences* 10(1), 800-811. https://doi.org/10.1080/2314808X.2023.2283268

Sonneville-Aubrun, O., Simonnet, J. T., and L'Alloret, F. (2004). "Nanoemulsions: A new vehicle for skincare products," *Advances in Colloid and Interface Science*, 108-109 145-149. https://doi.org/0.1016/J.CIS.2003.10.026

Tazimifar, F., Salavati-Niasari, M., Monsef, R. (2025). "Modulating electrochemical performance of La_2FeNiO_6/MWCNT nanocomposites for hydrogen storage inquiries: Schiff-base ligand-assisted synthesis and characterization." *Industrial & Engineering Chemistry Research* 64(36), 17294-17310. https://doi.org/10.1021/acs.iecr.5c01564

Tohamy, H. A. S., and El-Masry, H. M. (2024). "Fluffy-like amphiphilic graphene oxide and its effects on improving the antibacterial activity and thermal outstanding of ethyl cellulose /polyvinyl alcohol hydrogel film," *BMC Chemistry* 18(1), 1-9. https://doi.org/10.1186/S13065-024-01221-3/FIGURES/6

Ullah, I., Ullah, A., Rehman, S., Ullah, S., Ullah, H., Haqqni, S., Amir, M., Gul, F., and Bashir, K. (2023a). "Prevalence and risk factors of helicobacter pylori infection among individuals with tobacco consumption habits in district peshawar: A cross-sectional study," *Bulletin of Biological and Allied Sciences Research* 2023(1), article 42. https://doi.org/10.54112/bbasr.v2023i1.42

Ullah, W., Ullah, A., Khan, M., Hassan, N., Aman, K., Khan, S., Hassan, S., and Hazrat, A. (2023b). "Microbial profile and nutritional evaluation of broiler and domestic chicken meat from selected districts oF Khyber Pakhtunkhwa, Pakistan," *Bulletin of Biological and Allied Sciences Research* 2023(1), article 34. https://doi.org/10.54112/bbasr.v2023i1.34

Zhao, F., Li, Q., Wu, H., Huang, J., and Ju, J. (2023). "Synergistic antifungal mechanism of effective components from essential oil against *Penicillium roqueforti*," *Engineering Microbiology* 3(1), article 100057. https://doi.org/10.1016/J.ENGMIC.2022.100057

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