

Research Article

Lavender and Geranium Essential Oil-Loaded Nanogels with Promising Repellent and Antibacterial Effects

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Malaria and bacterial infections remain a life-threatening disease with a significant global impact. This study aimed to develop nanogels (NGs) of lavender (*Lavandula angustifolia* Mill.) and geranium (*Pelargonium graveolens* L'Hér.) essential oils (EOs) with promising repellent and antibacterial effects. The NGs were formulated using a nanoemulsion-based gel approach; nanoemulsion droplet sizes and zeta potentials were obtained as 146 ± 7 and 106 ± 6 nm and -23.2 ± 0.7 and -17.4 ± 1 mV, respectively. The ATR-FTIR analysis confirmed the successful loading of EOs in NGs. Repellent bioassays conducted on *Anopheles stephensi* Liston. mosquitoes demonstrated that geranium NG (140 min) was as effective as DEET (140 min), a widely used repellent. Antibacterial tests showed that the nanogels effectively reduced bacterial growth, with the geranium NG exhibiting over 90% reduction against *Escherichia coli* Migula. The lavender NG displayed higher efficacy against *Staphylococcus aureus* Rosenbach. These findings highlighted the potential of nanogels containing EOs as promising repellents and antibacterial agents, offering a sustainable and eco-friendly approach for vector control and bacterial infections.

1. Introduction

Malaria, a disease that poses a significant risk to human life, recorded a total of 247 million cases and 619,000 fatalities in the year 2021 [1]. The transmission occurs mainly through the bites of infected female *Anopheles* Meigen mosquitoes, which serve as vectors for the parasite [2]. *Anopheles stephensi* Liston. (Diptera: Culicidae) is an important malaria

vector in Iran, particularly in the southern and southeastern regions [3, 4]. Its distribution extends beyond Iran, with reports in countries within the Horn of Africa, including Djibouti, Somalia, Sudan, and Yemen [5]. Repellents play a crucial role in malaria control, particularly in endemic regions. The use of effective repellents not only safeguards individuals but also reduces the overall transmission of malaria in affected areas [6, 7]. DEET-based products are

widely used as repellents, although their side effects, such as skin and respiratory allergies, seizures, and acute manic syndrome, have caused their use to be questioned [8, 9].

Bacterial infections pose significant risks to human health and can become life-threatening if not promptly treated. *E. coli* Migula. and *S. aureus* Rosenbach. are two common examples of Gram-positive and negative bacteria that can cause severe infections [10, 11]. *S. aureus*, typically found on the skin and nose, can enter the body through wounds or surgical sites, leading to infections ranging from mild skin issues to more severe conditions like pneumonia or bloodstream infections. In extreme cases, it can cause life-threatening diseases such as endocarditis or necrotizing fasciitis [12]. *E. coli*, a normal resident of the intestines, can cause harmful infections when pathogenic strains contaminate food or cause urinary tract infections [13]. The excessive and improper use of antibiotics has led to the rise of antibiotic-resistant bacteria, underscoring the urgent need to develop new medications [14].

Essential oils (EOs) are aromatic oils that plants produce as secondary metabolites, known for their strong scent. These oils exhibit diverse biological properties, including larvicidal and repellent activities and antibacterial, antioxidant, and anticancer effects [15, 16]. For instance, lavender (*Lavandula angustifolia* Mill.) and geranium (*Pelargonium graveolens* L'Hér.) EOs or their extractions have been used for a long time as insect or mosquito repellents [17]. Several studies have investigated the chemical composition and biological properties of lavender EO. The EO was found to exhibit insecticidal activity and increase the percent repellency against insects [18, 19]. Additionally, lavender essential oil has been shown to have antioxidant, antimicrobial, and antifungal properties [20, 21]. Besides, geranium EO has been studied about various insects; e.g., it showed a repellent effect against adult flies, specifically *Musca domestica* and *Lucilia cuprina*. Another study highlighted the potential of geranium EO as a biopesticide for controlling insects [22, 23].

Despite the advantages of EOs, such as biocompatibility, biodegradability, and eco-friendliness, their volatility and oxidation ability should be improved [24]. Lately, there has been growing interest in preparing nanostructures containing EOs as a promising method to enhance their stability and effectiveness [25, 26]. Among these nanostructures, nanoemulsion-based gels with high drug loading capacity and suitable viscosity have emerged as viable dosage forms for developing topically administered products like repellents and antibacterial agents [27, 28].

The present study attempted to develop nanogels of lavender and geranium EOs with comparable efficacy to DEET against *Anopheles stephensi* Liston. Moreover, their antibacterial effects against *E. coli* and *S. aureus* were investigated.

2. Materials and Methods

2.1. Materials. *E. coli* Migula. (ATCC 25922) and *S. aureus* Rosenbach. (ATCC 25923) were bought from the Pasteur Institute of Iran. Mueller–Hinton agar, Mueller–Hinton

broth, carboxymethyl cellulose (CMC), and Tween 20 were purchased from Merck (Germany). Lavender and geranium EOs were purchased from Tabib Daru Company, Iran. They were extracted from the bark of plants through a water distillation process.

For this study, the Bandar-e-Abbas strain of *A. stephensi*, consisting of nulliparous female mosquitoes aged 5–8 days, was used. The mosquitoes utilized in this study were consistently bred for a period exceeding ten years within the insectary of Hormozgan University of Medical Sciences, located in Iran. It was noted that they were susceptible to insecticides. The mosquitoes were kept in ideal conditions, which involved maintaining a temperature of $27 \pm 2^\circ\text{C}$, relative humidity of $70 \pm 10\%$, and a light-dark cycle of 12 h (L:D).

2.2. Identification of EOs' Compounds. An Agilent GC-MS system with a 6890 Network GC System and a 5975 network mass selective detector was used to analyze the EOs. Separations were performed on an HP-5MS column using a specific temperature program described in our previous report [29].

2.3. Preparation and Characterizations of Nanogels. The nanoemulsion-based approach was used for the preparation of nanogels [30]. In brief, the mixture of each EO at a concentration of 2% w/v with Tween 20 at 4% w/v was stirred for 3 minutes at 2000 rpm at room temperature. Distilled water was then gradually added to the mixtures, up to a total volume of 20 mL, and stirred for 45 minutes to stabilize the nanoemulsions. The size characteristics of the nanoemulsions were analyzed using a Dynamic Light Scattering (DLS) device from K-One Nano Ltd, Korea. Nanoemulsions' droplet size distribution (SPAN) was calculated as the difference between the d90 and d10 droplet diameters divided by the d50 diameter, where d represents the droplet diameter and 90, 10, and 50 are percentages of droplets with smaller sizes than the specified points. Additionally, the zeta potentials of the nanoemulsions were examined using a zeta sizer from Horiba, SZ-100, Japan.

Subsequently, the nanoemulsions were mixed with CMC (3.5% w/v) and stirred overnight at 2000 rpm at room temperature to form nanogels. These nanogels were named lavender NG and geranium NG, respectively. As a negative control, a blank nanogel was prepared using the same process without adding any EOs. For comparison, DEET (2% w/v) was used as a positive control in the repellent bioassays, which was prepared by diluting DEET 40% from Reyhan Naghsh Jahan Pharmaceutical Co., Iran, with distilled water. To determine the viscosity of lavender NG and geranium NG, a rheometer machine (MCR-302, Anton Paar, Austria) was used at different shear rates ranging from 0.1 to 100 1/s and at room temperature. The Carreau–Yasuda model has been commonly used to describe the behavior of non-Newtonian fluids, where the viscosity decreases with increasing shear rate [26].

Furthermore, the loading of EOs in the nanogels was investigated using ATR-FTIR analysis. Spectra of lavender NG, geranium NG, EOs, and blank gels were recorded

within a wavenumber range of 400–3900 cm^{-1} . To assess the stability of lavender NG and geranium NG, these nanogels were stored at room temperature and in a refrigerator ($+4^{\circ}\text{C}$) for a period of six months. After the storage period, the nanogels were visually inspected for any changes, such as sedimentation, phase separation, or creaming [31].

2.4. Repellent Bioassay. Approximately 250 nulliparous 5–8 days aged female mosquitoes were nourished with non-blood food used for repellent tests. They were maintained in cages of $40 \times 40 \times 40$ cm size and not fed for 14 h before tests. The repellent tests were conducted using the arm-in-cage method. The main outcome of these tests was determining the complete protection time (CPT). Volunteer's forearms were first sterilized with 70% alcohol. Next, an $8 \text{ cm} \times 12.5 \text{ cm}$ area between the wrist and elbow (covered by fewer hairs) was treated separately with 1 g of DEET, lavender NG (contained 2% of the EO), and geranium NG (contained 2% of the EO). Besides, 1 g of blank gel was used as a negative control group. After 5 minutes, hands were exposed to mosquitoes in the cage for 3 minutes. This process was repeated for 30 minutes until one landing and/or biting happened in 3 minutes of exposure. In the last 30 minutes, the exposure to mosquitoes was continuous instead of 3 minutes to determine CPT more accurately.

2.5. Antibacterial Tests. ATCC100 assay was used to investigate the antibacterial properties of lavender NG and geranium NG. *E. coli* and *S. aureus* bacterial suspensions were prepared with 2×10^5 CFU/mL concentration in Mueller–Hinton broth media. Each suspension containing 4 mL of bacteria was filled into separate 5 cm plates. The antibacterial effects of lavender NG and geranium NG were tested at three different concentrations: 1250, 2500, and 5000 $\mu\text{g/mL}$. For comparison, bacteria without any treatment served as the control, and treatment with 1 g of blank gel acted as the negative control. The plates were then incubated at 37°C for 24 hours. After incubation, 10 μL of the bacterial suspensions from the plates was cultured on Muller–Hinton agar plates and incubated for another 24 hours. Finally, the number of bacterial colonies on the cultured plates was compared with the control group, and the growth of bacteria was calculated using the following equation: $\text{growth (\%)} = (\text{CFU sample} / \text{CFU control}) \times 100$. CFU represents the colony-forming unit, which indicates the number of viable bacterial cells.

2.6. Statistical Analyses. The experiments were conducted in triplicate, and the results were reported as mean $\pm$ standard deviation (SD). To assess the statistical significance of the data, one-way ANOVA and *T*-test were employed, with a significance level set at 0.05. These tests were used to compare two or more independent samples using STATA v11 software from StataCorp, USA.

3. Results

3.1. Compounds of Lavender and Geranium EOs. The compounds present in the EOs were identified using Gas Chromatography–Mass Spectrometry (GC–MS) analysis, and their details are provided in Table 1.

3.2. Prepared Nanoemulsion-Based Nanogels. Lavender EO nanoemulsion with a droplet size of 146 ± 7 nm and SPAN of 0.94 was prepared; its DLS profile is demonstrated in Figure 1(a). Besides, its zeta potential was -23.2 ± 0.7 mV, as shown in Figure 1(b). Moreover, a nanoemulsion of geranium EO was prepared, and its droplet size, SPAN, and zeta potential were obtained as 106 ± 6 nm, 0.97, and -17.4 ± 1 mV, respectively (Figures 1(c) and 1(d)).

The addition of CMC (3.5% w/v) resulted in the gelification of the nanoemulsions. The viscosity of the gelified nanoemulsions was analyzed at different shear rates (ranging from 0.1 to 100 1/s), and the data were well fitted with the Carreau–Yasuda model, as shown in Figures 2(a) and 2(b). Furthermore, the stability of both nanogels was assessed after six months of storage at room temperature and in a refrigerator ($+4^{\circ}\text{C}$). It was found that no sedimentation or phase separation was observed during this period, confirming the stability of the nanogels.

3.3. ATR-FTIR Analysis. ATR-FTIR spectra of lavender EO and geranium EO, blank nanogel, lavender NG, and geranium NG are shown in Figure 3. In the spectrum of lavender EO (Figure 3(a)), the absorption peak at around 3467 cm^{-1} corresponded to the O–H stretching vibrations. The peaks observed at 2966, 2925, 2876, 1449, 1368, and 1238 cm^{-1} were attributed to alkanes' C–H stretching. The absorption peak that appeared at 1737 cm^{-1} was related to C=O stretching. The bands located at 1044 and 1092 cm^{-1} might be related to the C–O vibration of alcohol, ethers, carboxylic acid, and ester.

In the spectrum of geranium EO (Figure 3(b)), various absorption peaks were observed at different locations, including at 3341, 2962, 2922, 2871, 1728, 1671, 1449, 1376, 1169, 1056, 1001, 831, and 742 cm^{-1} . The absorption peak at 3341 cm^{-1} corresponded to the O–H stretching vibration in the composition of geranium EO. The band observed at 2962 cm^{-1} was attributed to volatile substances' C=C–C ring vibrations. The major peaks at 2922 cm^{-1} and 2871 cm^{-1} could be related to the $-\text{CH}_2$, $-\text{C}-\text{H}$ asymmetric stretching and $-\text{CH}$, $-\text{C}-\text{H}$ symmetric stretching vibrations. The bands at 1728 and 1671 cm^{-1} were associated with carbonyl stretching vibrations (C=O). The significant peaks at 1449 and 1376 cm^{-1} were assigned to the CH_2 and CH_3 deformation vibrations. Furthermore, the major peak at 1169 cm^{-1} was attributed to the C–O stretching vibrations. The bands that appeared at 1001 and 831 cm^{-1} correspond to CH 's methylene vibrations and out-of-plane bending vibrations, respectively. Similar observations were also reported in the literature [32, 33].

In the spectrum of the blank gel (Figure 3(c)), a prominent peak was observed at approximately $3508\text{--}3698 \text{ cm}^{-1}$, corresponding to the OH group present in CMC. Other absorption bands, such as those at 2922, 1581, and

TABLE 1: Identified compounds in EOs by GC-MS analysis.

Compound	Lavender		Geranium		Retention index
	Area	%	Area	%	
Sabinene	—	—	2399926913	1.7	975
δ -3-Carene	—	—	5734932701	4.1	1008
2-Methylhexanoic acid	—	—	2463794364	1.7	1027
Limonene	—	—	5048955736	3.6	1029
β -Myrcene	36716766	1.0	—	—	988
Limonene	51639700	1.4	—	—	1024
1,8-Cineole	237873452	6.5	—	—	1026
<i>cis</i> -Ocimene	65867583	1.8	—	—	1037
<i>trans</i> -Ocimene	54037083	1.5	—	—	1050
Benzene ethanol	—	—	1558275791	1.1	1031
Menthone	—	—	5311708028	3.8	1152
Isomenthone	—	—	4353014742	3.1	1162
Linalool	889872802	24.4	12854571458	9.1	1095
Pelargol	—	—	5789332572	4.1	1196
Camphor	270512255	7.4	—	—	1146
Borneol	149523285	4.1	—	—	1169
4-Terpineol	65108952	1.8	—	—	1177
Hexenyl butanoate	97343196	2.7	—	—	1186
β -Citronellol	—	—	40708382043	28.8	1225
<i>trans</i> -Geraniol	—	—	15706284771	15.2	1254
Linalyl acetate	1142066219	31.3	—	—	1257
Lavandulyl acetate	84123640	2.3	—	—	1290
Geranyl butyrate	—	—	1403100749	1.0	1562
Geranyl acetate	38352298	1.1	—	—	1381
<i>trans</i> -Caryophyllene	89692079	2.5	—	—	1419
Diethyl phthalate	8874041326	6.3	8874041326	6.3	1587
Eudesmol	1549168817	1.1	1549168817	1.1	1619
Bulnesol	5292982976	3.7	5292982976	3.7	1671

1461 cm^{-1} , were also evident, attributed to the stretching vibrations of C-H, C=O stretching, and hydrocarbon groups ($-\text{CH}_2$), respectively. “Additionally, the peak observed at 1080–1252 cm^{-1} corresponded to the stretching of ether groups ($-\text{O}-$)” [34].

In the ATR-FTIR spectrum of lavender NG (Figure 3(d)), the changes in intensity and shape of several absorption peaks were recognized, which might be due to the interaction between lavender EO and CMC. For example, the absorption band at 3467 cm^{-1} attributed to $-\text{OH}$ in lavender EO was shifted to 3505 cm^{-1} in the spectrum of lavender NG. Moreover, the intensity of the absorption peak of the carbonyl group in the ATR-FTIR spectrum of lavender NG is significantly reduced compared to the spectrum of lavender EO; therefore, the loading of lavender EO into the nanogel was confirmed. Most of the peaks observed in the blank gel and geranium EO also exist in the spectrum of geranium NG (Figure 3(e)) with few shifts and changes in the shape and intensity of some peaks, so successful loading of the geranium EO was confirmed.

3.4. Nanogels’ Repellent Efficacy. The repellent efficacy of geranium NG 2% w/v with a complete protection time of 140 ± 6 min was equal to DEET (Figure 4). In addition, it was significantly more potent than lavender NG 2% w/v and blank gel, with complete protection times of 90 ± 5 and 10 ± 5 min ($P < 0.001$), respectively.

3.5. Nanogels’ Antibacterial Properties. The antibacterial effect of the lavender NG and geranium NG against *E. coli* is demonstrated in Figure 5. Interestingly, more than 90% of *E. coli* growth was reduced after treatment with 1250, 2500, and 5000 $\mu\text{g/mL}$ of geranium NG. Besides, 80, 40, and 0.5% bacterial growth was observed after treatment with 1250, 2500, and 5000 $\mu\text{g/mL}$ of lavender NG.

Moreover, the efficacy of both nanogels against *S. aureus* was less than that against *E. coli* (Figure 6). After treatment with 1250, 2500, and 5000 $\mu\text{g/mL}$ of lavender NG, bacterial growths were observed at 80, 70, and 50%, significantly less than geranium NG at equal concentrations (100, 90, and 85% bacterial growth). Besides, the blank gel did not affect bacterial growth.

4. Discussion

Vector-borne diseases such as chikungunya, malaria, Zika, yellow fever, West Nile fever, and Japanese encephalitis include 17% of all infectious diseases [35]. Vector-borne diseases in Iran have become a significant health concern due to global warming, globalization, and insecticide resistance [36]. The dispersion patterns of these diseases vary across different regions of Iran, with some diseases like Crimean-Congo hemorrhagic fever and Q fever being distributed throughout the country, while others like plague, leishmaniasis, tularemia, and malaria are restricted to specific areas [36, 37]. Leishmaniasis and Crimean-Congo

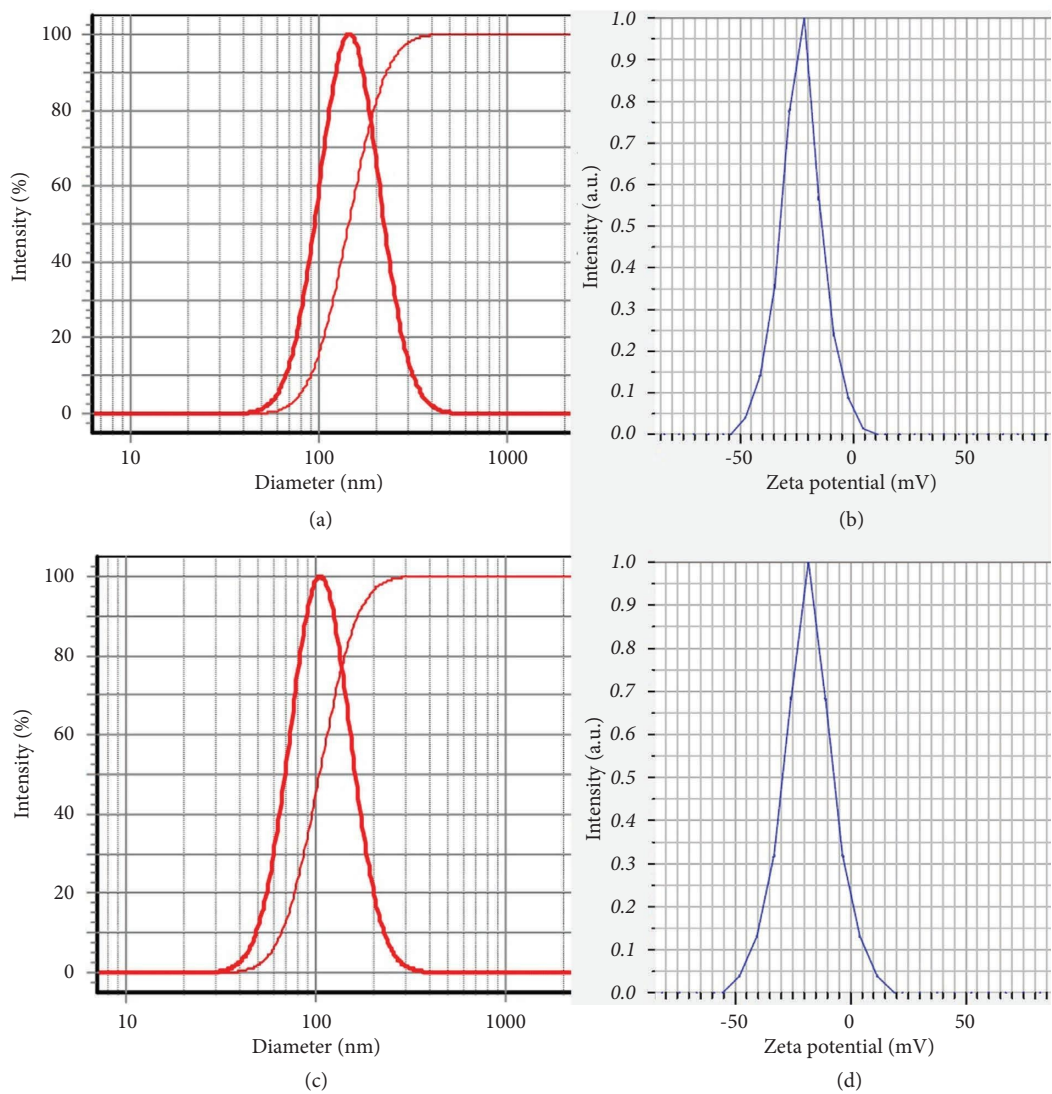


FIGURE 1: DLS and zeta potential analyses of the nanoemulsion of lavender EO (a, b) and nanoemulsion of geranium EO (c, d).

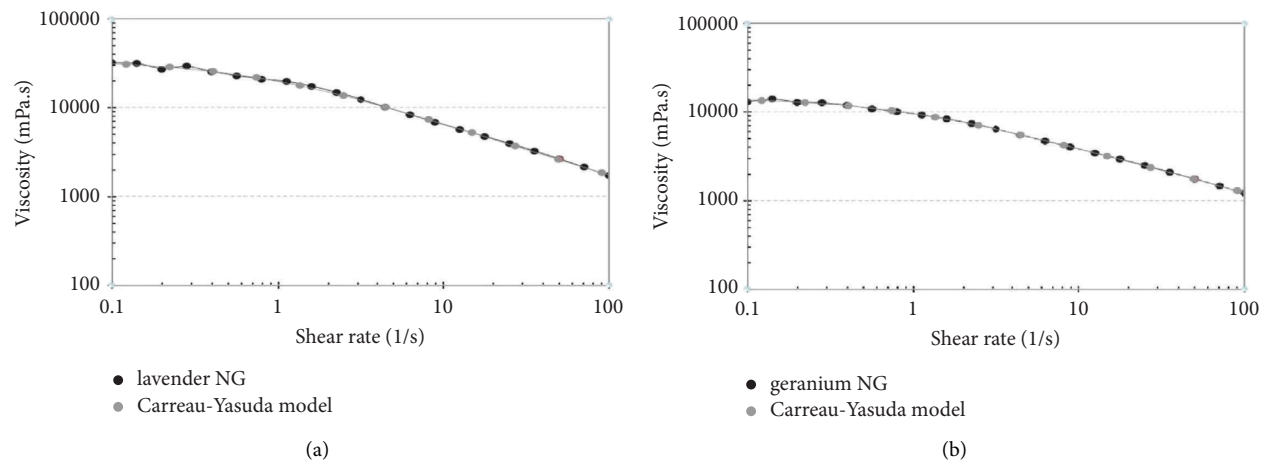


FIGURE 2: Viscosity of the nanogels of lavender EO (a) and geranium EO (b) at different shear rates.

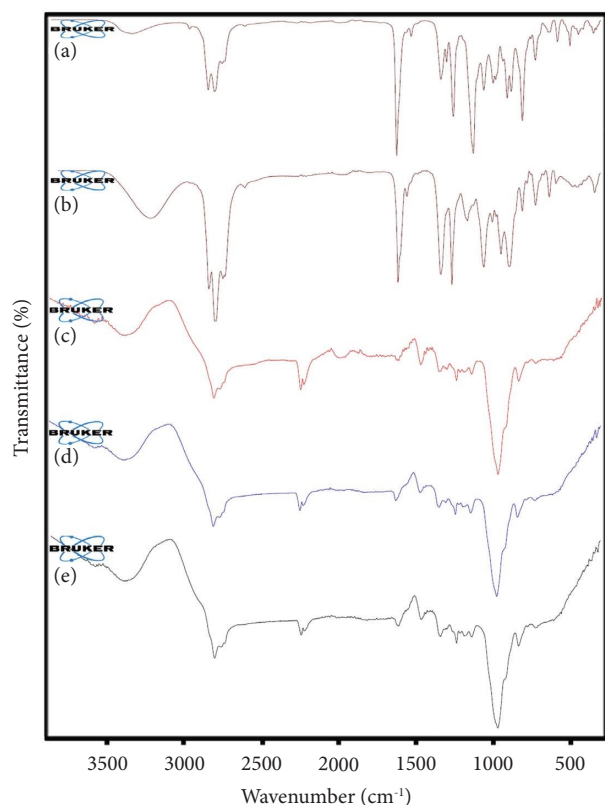


FIGURE 3: ATR-FTIR spectra of (a) lavender EO, (b) geranium EO, (c) blank gel, (d) lavender NG, and (e) geranium NG.

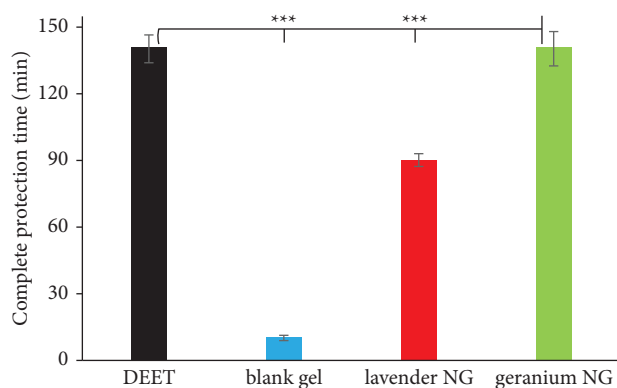


FIGURE 4: Protection times of lavender NG and geranium NG, blank gel (negative control), and DEET (positive control). The efficacy of geranium NG was more potent than lavender NG and blank gel ($***P < 0.001$).

hemorrhagic fever have been reported to have the highest incidence rates in Iran [38]. The COVID-19 pandemic has impacted the trend of vector-borne diseases, with some diseases experiencing a decrease in incidence [39].

In recent years, resistance to industrial insecticides and repellents has been one of the challenges to health systems. Researchers have thus been encouraged to develop natural insecticides and repellents [40, 41]. One of the effective strategies in controlling vector-borne diseases is repellents, substances that discourage arthropods from landing or

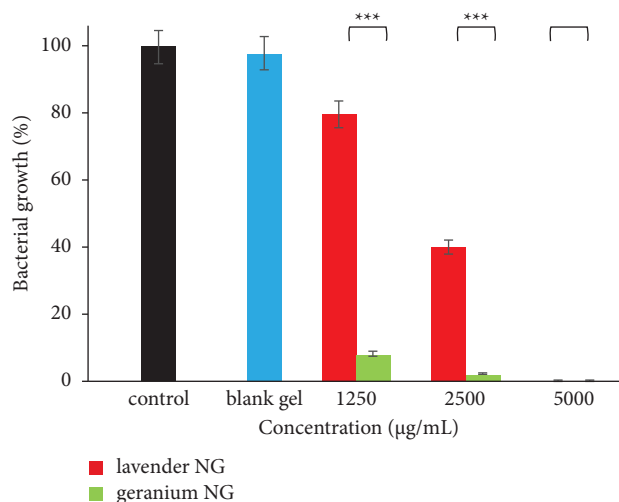


FIGURE 5: Antibacterial properties of lavender NG and geranium NG and blank gel (negative control) against *E. coli*. The control group was not exposed to any nanogel. $***P < 0.001$.

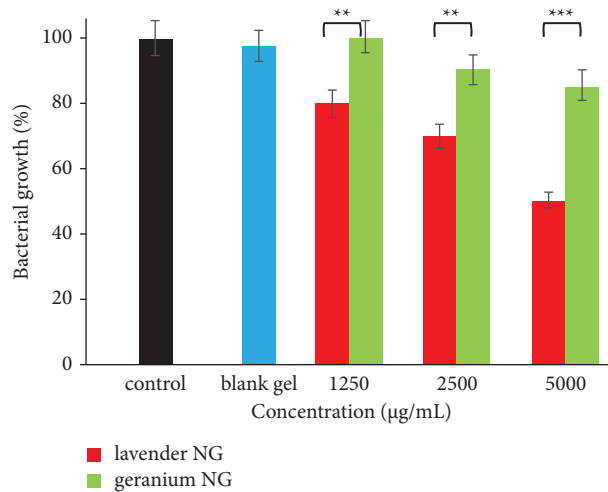


FIGURE 6: Antibacterial properties of lavender NG and geranium NG and blank gel (negative control) against *S. aureus*. The control group was not exposed to any nanogel. $***P < 0.001$ and $**P < 0.01$.

biting human skin [42, 43]. Carbon dioxide and lactic acid in human sweat are recognized by chemoreceptors in female mosquitoes' antennae [44]. Usually, insect repellents provide a vapor barrier deterring arthropods from coming into contact with the surface; EOs with volatile compounds and distinct odors have recently received more attention [45]. However, their volatility and efficacy should be improved. The best candidates for developing repellents are nanogels with soft tissue, flexibility, biocompatibility, good swelling ability, and structural stability [46, 47]. This study achieved comparable efficacy to DEET by formulating a nanogel containing *P. graveolens* EO (geranium NG).

Based on the current results, the obtained protection time for geranium NG (140 ± 6 min) was more than many reports in the literature. For instance, the efficacy of the nanogel containing *Mentha piperita* L. and *Foeniculum vulgare* Mill. EOs against *A. stephensi* was 120 ± 8 min and

70 ± 6 min [48]. Besides, solid lipid nanoparticles containing *Zataria multiflora* Boiss. showed 93 ± 5 min repellent effects against *A. stephensi* [49]. Moreover, the protection time of chitosan nanoparticles containing *Elettaria cardamomum* (L.) Maton against *A. stephensi* was 32 ± 4 min [50]. However, nanoformulations have been reported to have higher efficiency than the current study. For instance, nanoemulsion of 50% *M. piperita* EO showed a protection time of 257 min against *A. stephensi* [51]. Besides, nanoemulsion containing 50% *Eucalyptus globulus* Labill. EO revealed a protection time of 351 min against *A. stephensi* [51].

Bacterial infections pose significant health risks to humans and animals, leading to severe diseases. These infections are caused by harmful bacteria that can increase and harm the host's body, requiring prompt and appropriate medical attention to prevent further complications [52, 53]. EOs, as natural antibacterial agents, could be used to control bacterial diseases [54, 55]. Interestingly, more than a 90% reduction in the growth of *E. coli* was observed after treatment with geranium NG in the current study. The bioactivity of the geranium EO against bacteria might be mainly attributed to citronellol, linalool, and menthone; their antibacterial effect on *S. aureus* has been reported in the literature [56, 57].

In the current study, lavender NG showed more potency than geranium NG against *S. aureus*. Antimicrobial effects of lavender EO compounds such as linalyl acetate, linalool, 1,8-cineole, borneol, and camphor against *Rhyzopertha dominica* and *Sitophilus oryzae* were previously reported [58]. Gram-negative bacteria are typically more resilient to antibacterial agents compared to Gram-positive bacteria, primarily due to their outer membrane. However, EOs are known to exhibit selective effects, showing a preference for targeting either Gram-positive or Gram-negative bacteria [59]. As an example, *Myrtus communis* EO showed an IC₅₀ value of 4547 µg/mL against *E. coli*, but it exhibited significantly higher potency against *S. aureus* with an IC₅₀ value of 394 µg/mL [30]. Similarly, *M. piperita* EO demonstrated an IC₅₀ value of 27 mg/mL against *S. aureus*, yet it displayed greater potency against *E. coli* with an IC₅₀ value of 18 mg/mL [60]. On the other hand, small hydrophilic antibacterial agents like nanogel can pass through the outer membrane's porins [61, 62]. So, by preparing nanostructures-loaded EOs, their efficacy against Gram-negative bacteria could be improved; however, more studies are needed to select the appropriate EO.

5. Conclusions

Nanogels containing lavender and geranium EOs (2% w/v) were successfully developed with promising repellent and antibacterial effects. The geranium NG (2% w/v) demonstrated comparable repellent efficacy to DEET against *A. stephensi*. Additionally, both nanogels (2% w/v) effectively reduced bacterial growth, with the lavender NG showing higher efficacy against *S. aureus* and the geranium NG against *E. coli*. These results indicated that EO-loaded nanogels had the potential to serve as effective and

environmentally friendly options for vector control and management of bacterial infections.

Data Availability

All data are available upon reasonable request.

Ethical Approval

Fasa University of Medical Sciences has ethically approved the study, IR.FUMS.REC.1401.171. Moreover, all tests were carried out according to WHO and international guidelines. Besides, the process of the repellent bioassays was fully described to candidates; a 45-year-old man volunteered to participate in the current study. The corresponding author supported him for side effects during the study and one month later. However, no side effects were observed.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

ASD performed repellent assays. AA performed antibacterial tests. ZM and MRCh reviewed the literature and revised the manuscript. MS interpreted ATR-FTIR spectra. MO designed the study, prepared the nanoformulations, and analyzed the data. All authors contributed to the drafting of the manuscript and approved the final version.

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