Hindawi
Psyche: A Journal of Entomology
Volume 2022, Article ID 9991238, 8 pages
https://doi.org/10.1155/2022/9991238

Research Article

Larvicidal Effects of Nanoliposomes Containing Clove and Cinnamon Essential Oils, Eugenol, and Cinnamaldehyde against the Main Malaria Vector, *Anopheles stephensi* Liston

Alireza Sanei-Dehkordi,[1,2] Roghayeh Heiran,[3] Ghazaal Roozitalab,[4] Narges Elahi,[5] and Mahmoud Osanloo[6]

[1]*Department of Medical Entomology and Vector Control, School of Health, Hormozgan University of Medical Sciences, Bandarabbas, Iran*
[2]*Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandarabbas, Iran*
[3]*Department of Chemistry, Estahban Higher Education Center, Estahban 7451944655, Iran*
[4]*Noncommunicable Disease Research Center, Fasa University of Medical Sciences, Fasa, Iran*
[5]*Department of Tissue Engineering, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fasa, Iran*
[6]*Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fasa, Iran*

Correspondence should be addressed to Mahmoud Osanloo; osanloo_mahmood@yahoo.com

Received 18 December 2021; Accepted 6 September 2022; Published 16 September 2022

Academic Editor: Cleber Galvão

The use of larvicides, especially in endemic regions, is recommended for malaria control. However, due to the excessive use of synthetic larvicides, resistance in mosquitoes and environmental pollution have been challenges. In the current study, nanoliposome containing clove and cinnamon essential oils and their major ingredients, i.e., eugenol and cinnamaldehyde, were first prepared; particle size and successful loading were investigated using DLS (Dynamic Light Scattering) and ATR-FTIR (Attenuated Total Reflection-Fourier Transform InfraRed) analysis. Larvicidal effects of the nanoliposomes and nonformulated samples were then investigated against *Anopheles stephensi*. The best-observed efficacy (LC_{50} 5.4 μg/mL) was related to nanoliposomes containing eugenol with a particle size of 109 ± 4 nm. However, LC_{50} values of the other three nanoformulations were also around 10 μg/mL; all four prepared nanoformulations were thus introduced as natural larvicides for further investigations in the field conditions.

1. Introduction

Mosquitoes (*Diptera*: *Culicidae*) transmit malaria, dengue, yellow fever, encephalitis, filariasis, chikungunya, and Zika virus [1, 2]. Around 30 species from 400 identified *Anopheles* mosquito species are the vector of malaria to humans [3]. *Anopheles stephensi* Liston. is one of the most important malaria vectors in the Middle East and South Asia; however, it has recently expanded to Ethiopia, Djibouti, Lakshadweep, and Sri Lanka [4, 5]. Larvae are the weakest members in the life cycle of mosquitoes; the use of larvicides is thus recommended to control malaria transmission, especially in endemic regions [6, 7]. However, the excessive use of synthetic larvicides has led to widespread resistance or intolerance, adverse environmental risks, and side effects on human health or other nontarget species [8, 9].

Aromatic plants generate secondary metabolites known as essential oils (EOs), with various biological effects such as larvicidal and repellent effects. For instance, *Syzygium aromaticum* (L.) Merr. & L.M.Perry (clove) and *Cinnamomum zeylanicum* Blume (cinnamomum) are two medicinally important plants; their EOs possess larvicidal effects [10, 11]. The EOs with distinct properties such as ecocompatibility, biodegradability, and biocompatibility are

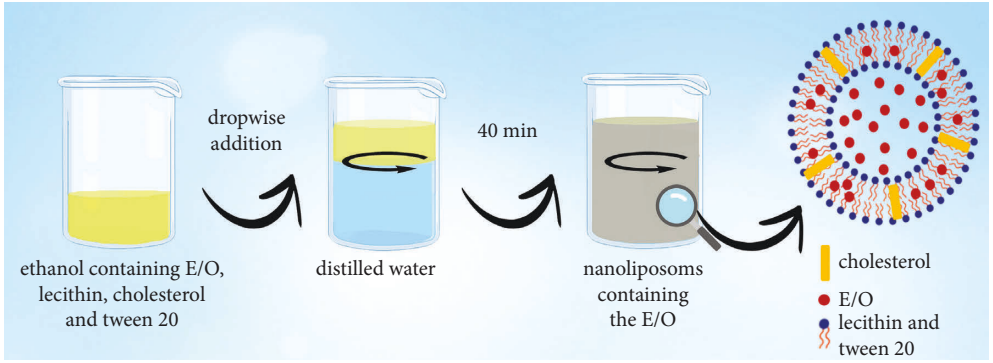

Figure 1: Preparation of nanoliposome containing eugenol, clove EO, cinnamaldehyde, and cinnamon EO.

proposed as proper alternatives for synthetic ones [12, 13]. However, the use of EOs as larvicides is hampered by their water immiscibility, high volatility, heating, swift oxidation, and degradation of on-air exposure [14, 15]. The preparation of EO-based nanoformulations has been recently proposed to meet the challenges [16]. Liposomes are minute vesicles comprising a lipid bilayer of amphiphilic molecules mimicking cells [17, 18]. Cargoes such as EOs or other natural larvicides could be entrapped into nanoliposomes to enhance stability, potency, efficacy, and durability [19, 20].

This study first investigated the larvicidal effects of clove and cinnamomum EOs and their major ingredients (eugenol and cinnamaldehyde) against *A. stephensi*. Then, an attempt was made to improve their efficacy by preparing nanoliposomes containing each.

2. Materials and Methods

2.1. Materials. All commercially available compounds were used as received. Wool fat cholesterol, tween 20, egg yolk lecithin, cinnamaldehyde, eugenol, and absolute ethanol were obtained from Merck Chemicals Co. (Germany). Cinnamon and clove EOs were purchased from Zardband Pharmaceuticals Co. and Green Plants of Life Co. Ltd. (Iran), companies with proprietary areas to grow the medicinal plants. This research used the late third and early fourth instar larvae of *A. stephensi* (Bandar-e-Abbas strain); they were supplied from the Hormozgan University of Medical Sciences (Iran). All colonies were reared and maintained under the recommended conditions; 27 ± 2°C, 65 ± 5% relative humidity, in 12L : 12D h photoperiod (L: light, D: dark). We used the polytetrafluoroethylene (PTFE)-based membrane method for blood-feeding adult female mosquitoes [21].

2.2. Preparation of Loaded Nanoliposomes. The nanoliposomes containing EOs, eugenol, and cinnamaldehyde were prepared by the ethanol injection method [22]. The process of preparing loaded nanoliposomes is illustrated in Figure 1. Lecithin (3% w/v), cholesterol (1.0% w/v), tween 20 (0.5%), and each of eugenol, clove EO, cinnamaldehyde, and cinnamon EO (2.0% w/v) was first fully dissolved in absolute ethanol at room temperature overnight (2000 rpm). After that, 1 mL of the obtained mixture was added dropwise to 4 mL of distilled water (2000 rpm). The mixture was kept under stirring conditions for 40 minutes to stabilize formed nanoliposomes. The prepared samples were abbreviated as eugenol-lipo, clove-lipo, cinnamaldehyde-lipo, and cinnamon-lipo.

2.3. Size Characterization. The mean diameter and particle size distribution (SPAN) of all nanoliposomes were investigated using a dynamic light scattering (DLS) instrument (K-One Nano, Ltd, Korea). In addition, the SPAN of the samples was also calculated by the equation d90—d 10/d50. Where d is diameter and 90, 10, and 50 are percentile of particles with lower diameter than these values.

2.4. Investigation of Loading of EOs, Eugenol, and Cinnamaldehyde in the Nanoliposomes. The Attenuated Total Reflection-Fourier Transform InfraRed (ATR-FTIR) was investigated to investigate the successful loading of eugenol, clove EO, cinnamaldehyde, and cinnamon EO into nanoliposome. Before being subjected to the analysis, the free nanoliposome and each loaded one was centrifuged for 60 min at 12000 g (4°C). The obtained pellets were stored at room temperature for three days to reduce their moisture. The spectra of each sample in the 400 to 4000 cm^{-1} were then recorded by a spectroscopy apparatus (Bruker Company, Model Tensor II, Germany).

2.5. Larvicidal Bioassays. Eugenol, clove EO, cinnamaldehyde, and cinnamon EO were dissolved in ethanol at a concentration of 2.0% w/v, equal to the concentration of the prepared nanoliposomes. Larvicidal effects of non-formulated and nanoformulated samples (eugenol-lipo, clove-lipo, cinnamaldehyde-lipo, and cinnamon-lipo) were investigated in line with the WHO guidelines [9]. Briefly, by adding different amounts (31.3, 62.5, 125, 250, 500, and 1000 μL) of the samples to batches of *A. stephensi* larvae (25 n in 200 mL dechlorinated water), their concentration was fixed at 100, 50, 25, 12.50, 6.25, and 3.13 μg/mL. After 24 h exposure, larval mortality was calculated; larvae with no response to stimulation with a probe were considered dead.

2.6. Statistical Analyses. Larvicidal bioassay was carried out in triplicate, and larval mortality was presented as mean ± standard deviation. Calcusyn software (free

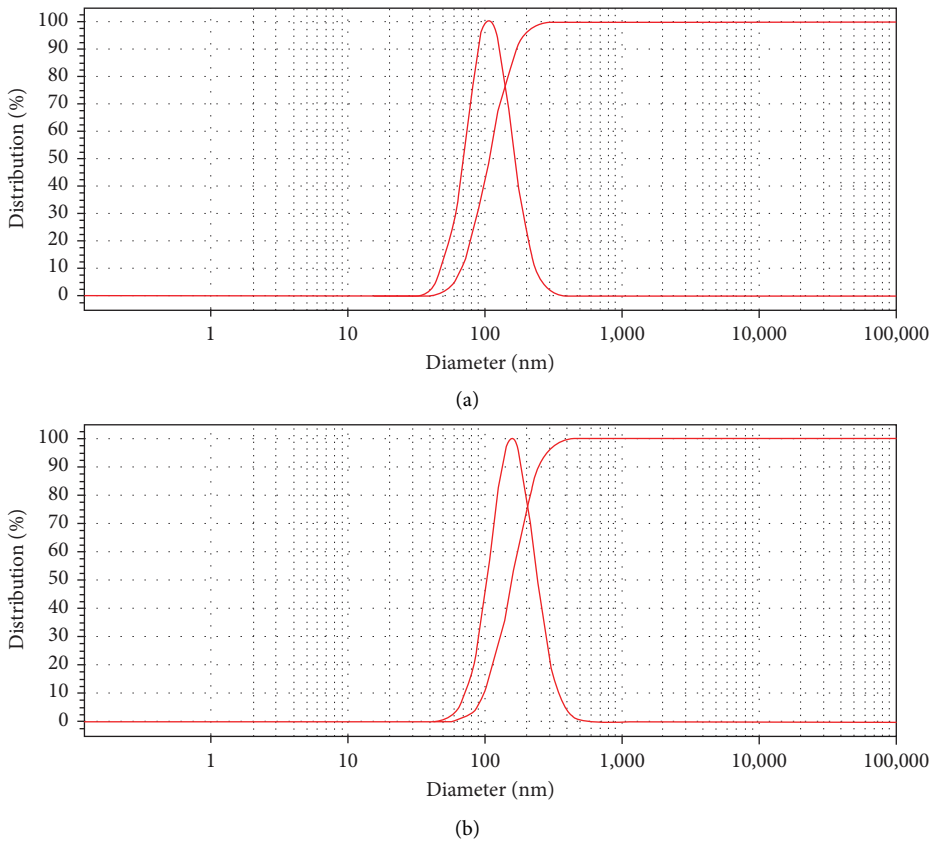

Figure 2: DLS profile of (a) eugenol-lipo 109 ± 4 nm (SPAN 0.96), (b) clove-lipo 158 ± 4 nm (SPAN 0.96).

version, BIOSOFT Co., UK) was used to calculate LC_{50} values of all samples with their upper and lower confidence interval (95%). The nonoverlap between the samples' upper and lower limit values was interpreted as statistically significant.

3. Results

3.1. Particles Size of the Prepared Nanoliposomes. DLS profiles of the eugenol-lipo and clove-lipo with particle sizes of 109 ± 4 and 158 ± 4 nm are depicted in Figure 2. Besides, their SPAN values were calculated as 0.96 and 0.96. Moreover, particle sizes of the cinnamaldehyde-lipo and cinnamon-lipo were obtained as 111 ± 6 and 195 ± 9 nm, and SPAN values were 0.96 and 0.97 (Figure 3). SPAN values of all mentioned formulations were lower than 1, so their narrow particle size distribution was confirmed [23].

3.2. Confirming Loading in the Liposomes. ATR-FTIR spectroscopy is one of the most useful methods to detect whether the EOs or major compounds were successfully incorporated into the liposome. The ATR-FTIR spectra of free liposome (Figure 4(a)), eugenol-lipo (Figure 4(b)), clove-lipo (Figure 4(c)), cinnamaldehyde-lipo (Figure 4(d)), and cinnamon-lipo (Figure 4(e)) are shown in Figure 4.

The free liposome spectrum showed the C-H and C-O stretching modes at 2980–2904 and 1044 cm^{-1}. The bands at 1453–1274 cm^{-1} were ascribed to the bending modes of CH_2, CH_3, and COH, and the absorption signals at 1274–877 cm^{-1} were attributed to C-N and PO bonds phospholipid.

From the eugenol-lipo spectrum (Figure 4(b)), the hydroxy stretching (liposome and eugenol) at around 3377 cm^{-1} and C-H stretching (liposome and eugenol) at 3004, 2923, and 2852 cm^{-1} could be clearly observed. The band ascribed to the stretching vibration of the carbonyl group of liposomes observed at 1710 cm^{-1}, and the absorptions at 1638, 1612, 1513, and 1464 cm^{-1} were attributed to C=C stretching vibrations. The sharp band at 1513 cm^{-1} was assigned to aromatic C=C stretching of eugenol. The bending mode of CH_2 and CH_3 appeared at 1431 and 1367 cm^{-1}, and the new absorption bands at 1267–1034 cm^{-1} were attributed to C-O stretching modes of eugenol. Moreover, the bending modes of CH and C=C and the vibrations of C-N, P=O, and P-O were observed at 1367–647 cm^{-1}. From the results, we could confirm the loading of eugenol into liposomes.

Nanoliposome with the addition of clove EO (clove-lipo) exhibited similar major peaks of liposome and clove EO (Figure 4(c)). The absorption bands of eugenol were prominent in the spectrum of clove-lipo. The broadband at 3350 cm^{-1} and the bands at 3004, 2923, and 2852 cm^{-1} signified O-H and C-H stretch. The bands at 1732 and 1712 cm^{-1} correspond to carbonyl groups, the strong band at 1514 cm^{-1} is due to aromatic C=C absorption, and the other C=C vibrations appeared at 1638–1463 cm^{-1}. The bands at

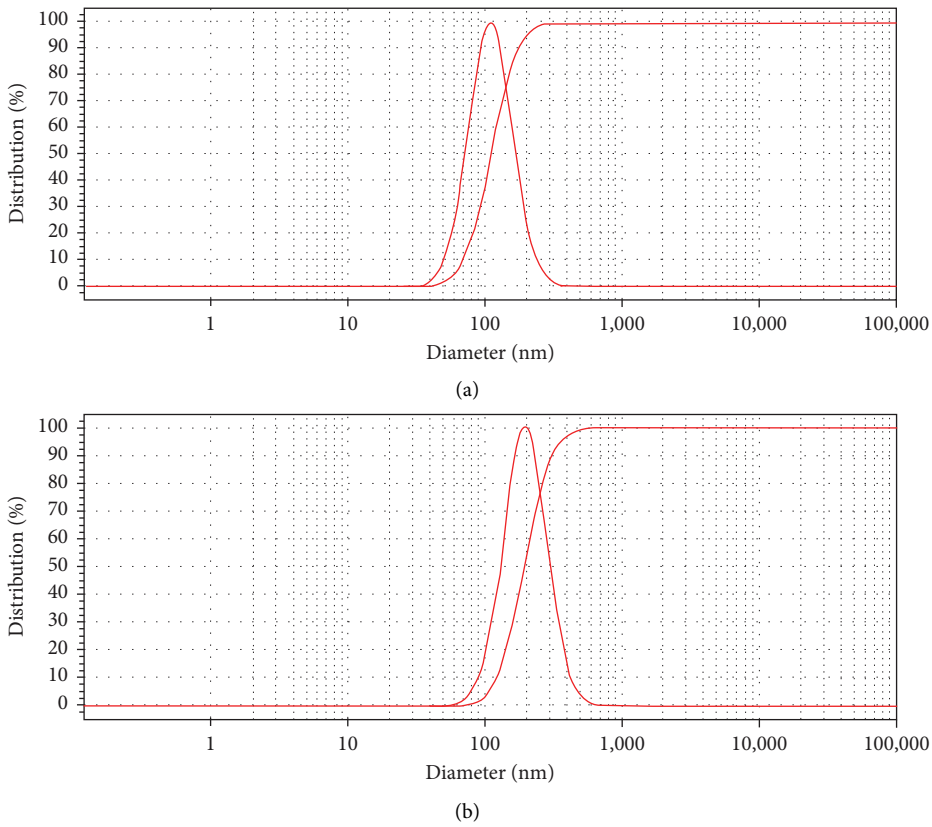

Figure 3: DLS profile of (a) cinnamaldehyde-lipo 111 ± 6 nm (SPAN 0.96) (b) cinnamon-lipo 195 ± 9 nm (SPAN 0.97).

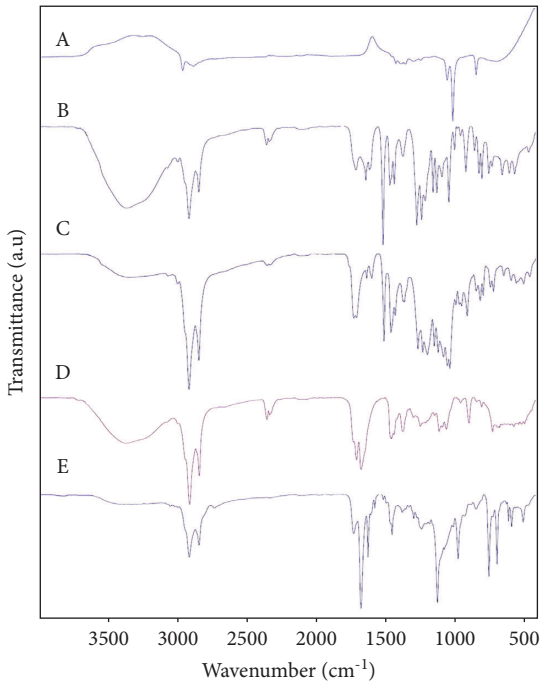

Figure 4: ATR-FTIR analyses of (a) free liposomes, (b) eugenol-lipo, (c) clove-lipo, (d) cinnamaldehyde-lipo, and (e) cinnamon-lipo.

1432 and 1366 cm^{-1} result from the bending vibration of CH_2 and CH_3. The wavenumbers of 1268–1037 cm^{-1} represented the vibrations of C-O and C-N bonds, and the absorption of P-O and P=O appeared at 1268–721 cm^{-1}. Overall, the results indicated that clove EO was successfully loaded into liposomes.

When cinnamaldehyde was incorporated into the liposome (Figure 4(d)), the spectra represented peaks at 3390 and 1708 cm^{-1} related to hydroxy and carbonyl moieties, which is the characteristic peak of the liposome. The strong peak at 2922 cm^{-1} approved the presence of CH bonds in liposome and cinnamaldehyde, CO-H, and carbonyl stretching vibrations of cinnamaldehyde were located at 2851 and 1676 cm^{-1}, respectively. The characteristic peaks at 1676–1372 cm^{-1} were attributed to stretching modes of C=C, and bending modes of CH_2 and CH_3 groups in cinnamaldehyde and liposome. The bands between 1296-1056 cm^{-1} indicated the presence of C-O (both EO and liposome) and PO_2^- bonds (in liposome), while the vibration of $(CH_3)_3N^+$ was located around 952 and 892 cm^{-1}, respectively. All of which confirm the existence of cinnamaldehyde in the liposome.

The ART-FTIR characteristic peaks for cinnamon-lipo have been illustrated in Figure 4(e). A broad peak around 3338 cm^{-1} showed the presence of hydroxy groups for alcoholic and phenolic hydroxy groups of cinnamon fractions and liposomes. The band at 2923 cm^{-1} indicates a C-H stretch in both cinnamon and liposome, and the absorbance

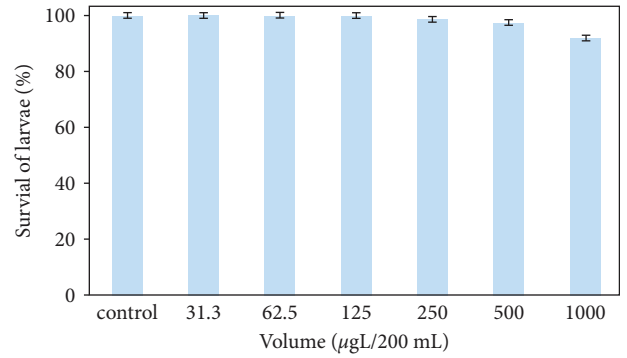

FIGURE 5: Larvicidal effects of free liposomes at different amounts in the larvicidal tests.

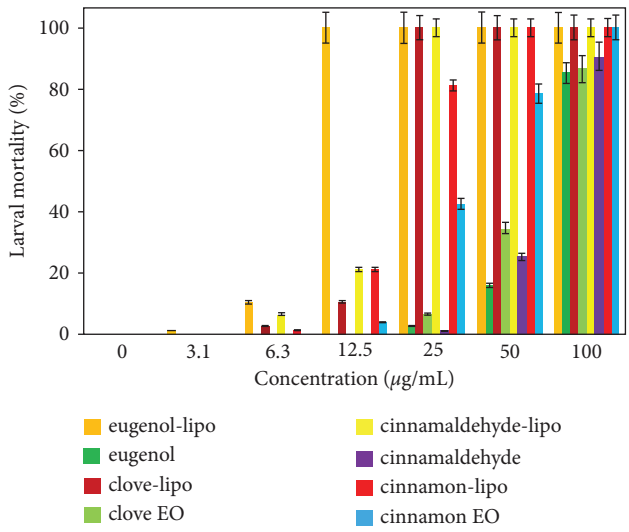

FIGURE 6: Larvicidal effects of samples at different concentrations against *A. stephensi*.

bands at 2852 and 2739 cm^{-1} revealed the presence of the CO-H bond for aldehydes. The peak at 1730 cm^{-1} was recognized due to the carbonyl group of liposomes. Besides this, the peaks at 1675 and 1625 cm^{-1} may be attributed to the stretching vibration of carbonyl groups corresponding to cinnamaldehyde and other aldehydes in cinnamon EO. Furthermore, the bands between 1606-1377 cm^{-1} correspond to C=C stretching modes and C-H bending modes of methyl and methylene moieties. The strong absorption at 1121 cm^{-1} was due to the stretch of C-O bonds, and the other characteristic peaks from 1328 to 688 cm^{-1} were assigned for vibrations of C-N, C-O, P=O, and P-O bonds, deformation of COH, bending modes of CH, as well as the long-chain band.

3.2.1. Larvicidal Effects of the Samples. Larvicidal effects of free liposomes at different amounts in the larvicidal test are depicted in Figure 5. These amounts were equal to the values used to reach the examined concentrations of the samples containing the EO (eugenol-lipo, clove-lipo, cinnamaldehyde-lipo, and cinnamon-lipo); only 8% of larvae survival was reduced at the highest amount (1 mL). Besides, larvicidal effects of all samples, including nonformulated samples and nanoformulations at a concentration range of 0–100 μg/mL, are shown in Figure 6. Interstingly, eugenol-lipo at 12.5–100 μg/mL concentrations showed perfect efficacy (caused 100% larvicidal effect).

Furthermore, obtained LC_{50} values of samples against *A. stephensi* are shown in Figure 7. Eugenol-lipo with an LC_{50} value of 5.37 (3.2–8.8) μg/mL showed the best efficacy; its LC_{50} was significantly more potent ($p < 0.05$) than eugenol, clove EO, cinnamaldehyde, cinnamon-lipo, and cinnamon EO. Besides, LC_{50} values of three other nanoformulations, including clove-lipo, cinnamaldehyde-lipo, and cinnamon-lipo, were obtained as 10.5 (6.2–17.9), 9.8 (5.6–17.1), and 13.7 (9.3–20.3) μg/mL. Moreover, LC_{50} values of nonformulated samples, including eugenol, clove EO, cinnamaldehyde, and cinnamon EO, were observed as 67.6 (55.3–82.6), 57.7 (53.6–62.1), and 62.2 (61.4–63.1) μg/mL.

4. Discussions

Chemical compositions of the used clove and cinnamon EOs in the current study have been investigated in our previous studies using Gas Chromatography-Mass Spectrometry analysis. As a result, thirty-three constituents were identified in clove EO; eugenol (65.41%), *trans*-caryophyllene (12.06%), eugenol acetate (9.85%), caryophyllene oxide (3.00%), and α-humulene (1.73%) were its five major constituents [24]. Besides, thirty constituents were identified in cinnamon EOs with five major compounds, including cinnamaldehyde (62.04%), linalool (6.96%), *trans*-caryophyllene (6.60%), *trans*-cinnamyl acetate (4.29%), and benzyl benzoate (3.32%) [25].

Nanoliposomes are tiny spherical vesicles (diameter <200 nm) spontaneously formed by phospholipids bilayer membrane in an aqueous medium [26, 27]. Hydrophobic materials such as eugenol and cinnamaldehyde are more loaded in the membrane; however, EOs (e.g., clove and cinnamon), a mixture of hydrophobic/hydrophilic substances, are loaded both in the membrane and central aqueous cavity [17, 19]. In the current study particle size of the liposomes containing eugenol and cinnamaldehyde was smaller than the liposomes containing clove and cinnamon EO, probably due to the loading of larger amounts EOs' compounds into the central cavity.

The loading process improves the physicochemical stability of the cargoes as pesticides and prevents the degradation of active agents [28, 29]. In addition, nanocarriers containing cargo provide a controlled release at the site of action, and thus their efficacy periods are longer [30, 31]. Moreover, particles with nanoscale allowed entering larvae bodies pores; consequently, the spreading of cargoes improves [32, 33]. Besides, when a solute such as EO is dissolved in a solvent, its droplet size is less than ~1 nm [34, 35]. Therefore, inside a nanoparticle with a diameter of 200 nanometers, around 1 million drops could be loaded; the packages containing EO reach the larval body [16, 36]. As a result, the bioactivity and efficacy of EO-based nanoformulations are generally higher than those of free EOs [37, 38].

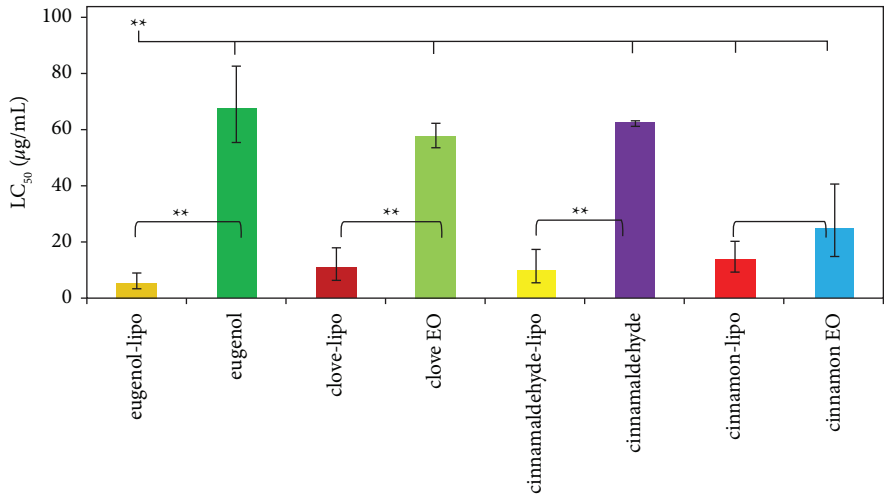

Figure 7: Obtained LC_{50} value of samples against *A. stephensi*; $^{**}p < 0.05$.

In the current study, LC_{50} values of eugenol and cinnamaldehyde were improved compared with their nanoliposomal states 12 and 6 folds. This improvement for clove and cinnamon EOs versus liposomes containing them was observed as 6 and 2 folds. Reviewing the literature, size-dependent improvements are not limited to the current study. For instance, the larvicidal effects of limonene and three limonene reach EOs from the *citrus* family, including *C. aurantium*, *C. limon*, and *C. sinensis* against *A. stephensi* were investigated; LC_{50} values were obtained as 20, 62, 13, and 12 μg/mL. Their nanoliposomal state with LC_{50} values of 13, 6, 6, and 9 was significantly more potent than the nonformulated states [22]. In another paper, larvicidal effects of carvacrol and two carvacrol reach EOs, including *Satureja khuzestanica* and *Zataria multiflora* EO against *A. stephensi,* were investigated; LC_{50} values were obtained as 128, 42, and 79 μg/mL. However, their nanoliposomal states with LC_{50} values of 11, 12, and 10 μg/mL were significantly more potent than nonformulated states [39]. Interestingly, the potency of eugenol-lipo with an LC_{50} value of 5 μg/mL against *A. stephensi* was more potent than the mentioned reports.

Furthermore, the LC_{50} value of chitosan nanoparticles containing cinnamon EO was obtained as 2.98 μg/mL in our previous study [11]; however, the LC_{50} value of nanoliposomes containing cinnamon EO was achieved at 13.7 μg/mL in the current study. Besides, the larvicidal effect of free chitosan nanoparticles in the mentioned report and another report by our team were around 18% [11, 40]. While free liposomes in the current study did not show a significant larvicidal effect, one of the differences in the result of that study with the present study is thus the difference in the nanocarriers used. Moreover, liposomes have an advantage over chitosan nanoparticles due to their high loading capacity for EO or their main constituents. However, more research is needed at the same time for these carriers.

5. Conclusions

The current study used eugenol, clove EO, cinnamaldehyde, and cinnamon EOs as natural larvicides against the main malaria vector mosquito, *A. stephensi*. An attempt was made to improve their efficacy by preparing nanoliposomes containing each; interestingly, their efficacy (LC_{50} values~10 μg/mL) about 2–12 folds was improved. The prepared nanoliposomes were introduced as natural larvicides for further investigations and against other medically critical mosquitoes.

Data Availability

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Ethical Approval

This research did not involve *in vivo* or human study, so no consent form was used. Besides, it has been ethically approved by the ethical committee of Fasa University of Medical Sciences, IR.FUMS.REC.1399.031. Moreover, all methods in the current study were performed according to the WHO (World Health Organization) relevant guidelines and national regulations.

Consent

Ok.

Conflicts of Interest

Researchers have no conflicts of interest in this study.

Authors' Contributions

ASD performed larvicidal tests. RH interpreted ATR-FTIR spectra. GhR and NE reviewed the literature. 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.

Acknowledgments

Fasa University of Medical Sciences funded this study, grant No. 97502.

References

[1] L. R. Bowman, S. Donegan, and P. J. McCall, "Is dengue vector control deficient in effectiveness or evidence?: systematic review and meta-analysis," *PLoS Neglected Tropical Diseases*, vol. 10, no. 3, Article ID e0004551, 2016.

[2] L. H. Franklinos, K. E. Jones, D. W. Redding, and I. Abubakar, "The effect of global change on mosquito-borne disease," *Lancet Infectious Diseases*, vol. 19, no. 9, pp. e302–e12, 2019.

[3] WHO, "Malaria fact sheet," 2021, https://www.who.int/news-room/fact-sheets/detail/malaria.

[4] S. N. Surendran, K. Sivabalakrishnan, A. Sivasingham et al., "Anthropogenic factors driving recent range expansion of the malaria vector *Anopheles stephensi*," *Frontiers in Public Health*, vol. 7, 2019.

[5] M. Oshaghi, F. Yaaghoobi, H. Vatandoost, M. Abaei, and K. Akbarzadeh, "*Anopheles stephensi* biological forms; geographical distribution and malaria transmission in malarious regions of Iran," *Pakistan Journal of Biological Sciences*, vol. 9, no. 2, pp. 294–298, 2006.

[6] S. Senthil-Nathan, "A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes," *Frontiers in Physiology*, vol. 10, p. 1591, 2019.

[7] M. Pani, G. Nahak, and R. K. Sahu, "Review on larvicidal activity of medicinal plants for malaria vector control," *International Journal of Current Pharmaceutical Review and Research*, vol. 6, no. 2, pp. 94–114, 2015.

[8] A. Amani, M. Osanloo, S. Amini, and M. Sedaghat, "Larvicidal activity of chemically synthesized silver nanoparticles against *Anopheles stephensi*," *Journal of Pharmaceutical Negative Results*, vol. 10, no. 1, pp. 69–74, 2019.

[9] WHO, *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*, WHO, Geneva, Switzerland, 2005.

[10] A. Manimaran, M. M. J. J. Cruz, C. Muthu, S. Vincent, and S. Ignacimuthu, "Larvicidal and knockdown effects of some essential oils against *Culex quinquefasciatus* Say, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston)," *Advances in Bioscience and Biotechnology*, vol. 3, no. 7, Article ID 24695, 2012.

[11] A. Sanei-Dehkordi, M. D. Moemenbellah-Fard, H. Sereshti, M. Shahriari-Namadi, E. Zarenezhad, and M. Osanloo, "Chitosan nanoparticles containing Elettaria cardamomum and cinnamomum zeylanicum essential oils; repellent and larvicidal effects against a malaria mosquito vector, and cytotoxic effects on a human skin normal cell line," *Chemical Papers*, vol. 75, pp. 6545–6556, 2021.

[12] A. Sanei-Dehkordi, S. Gholami, M. R. Abai, and M. M. Sedaghat, "Essential oil composition and larvicidal evaluation of platycladus orientalis against two mosquito vectors, *Anopheles stephensi* and *Culex pipiens*," *Journal of arthropod-borne diseases*, vol. 12, no. 2, pp. 101–107, 2018.

[13] M. Soleimani-Ahmadi, A. Sanei-Dehkordi, H. Turki et al., "Phytochemical properties and insecticidal potential of volatile oils from tanacetum persicum and achillea kellalensis against two medically important mosquitoes," *Journal of Essential Oil Bearing Plants*, vol. 20, no. 5, pp. 1254–1265, 2017.

[14] T. G. Thomas, S. Rao, and S. Lal, "Mosquito larvicidal properties of essential oil of an indigenous plant, ipomoea cairica linn," *Japanese Journal of Infectious Diseases*, vol. 57, no. 4, pp. 176-177, 2004.

[15] N. H. Hung, P. Satyal, H. V. Hieu et al., "Mosquito larvicidal activity of the essential oils of erechtites species growing wild in Vietnam," *Insects*, vol. 10, no. 2, 2019.

[16] F. Esmaili, A. Sanei-Dehkordi, F. Amoozegar, and M. Osanloo, "A review on the use of essential oil-based nanoformulations in control of mosquitoes," *Biointerface Research in Applied Chemistry*, vol. 11, no. 5, 2021.

[17] Z. Fakhravar, P. Ebrahimnejad, H. Daraee, and A. Akbarzadeh, "Nanoliposomes: synthesis methods and applications in cosmetics," *Journal of Cosmetic and Laser Therapy*, vol. 18, no. 3, pp. 174–181, 2016.

[18] M. Mozafari, *Nanoliposomes: Preparation and Analysis*, Springer, Berlin, Germany, 2010.

[19] M. Demirci, M. Y. Caglar, B. Cakir, and İ. Gülseren, "3-encapsulation by nanoliposomes," *Nanoencapsulation Technologies for the Food and Nutraceutical Industries*, pp. 74–113, 2017.

[20] P. Asadi, A. Mehravaran, N. Soltanloo, M. Abastabar, and J. Akhtari, "Nanoliposome-loaded antifungal drugs for dermal administration: a review," *Current Medical Mycology*, vol. 7, no. 1, pp. 71–78, 2021.

[21] D. J. Siria, E. P. A. Batista, M. A. Opiyo et al., "Evaluation of a simple polytetrafluoroethylene (PTFE)-based membrane for blood-feeding of malaria and dengue fever vectors in the laboratory," *Parasites & Vectors*, vol. 11, no. 1, p. 236, 2018.

[22] A. Sanei-Dehkordi, M. D. Moemenbellah-Fard, M. Saffari, E. Zarenezhad, and M. Osanloo, "Nanoliposomes containing limonene and limonene-rich essential oils as novel larvicides against malaria and filariasis mosquito vectors," *BMC Complementary Medicine and Therapies*, vol. 22, no. 1, 2022.

[23] N. Abedinpour, A. Ghanbariasad, A. Taghinezhad, and M. Osanloo, "Preparation of nanoemulsions of mentha piperita essential oil and investigation of their cytotoxic effect on human breast cancer lines," *BioNanoScience*, vol. 11, no. 2, pp. 428–436, 2021.

[24] M. D. Moemenbellah-Fard, A. Abdollahi, A. Ghanbariasad, and M. Osanloo, "Antibacterial and leishmanicidal activities of syzygium aromaticum essential oil versus its major ingredient, eugenol," *Flavour and Fragrance Journal*, vol. 35, no. 5, pp. 534–540, 2020.

[25] A. Ghanbariasad, A. Valizadeh, S. N. Ghadimi, Z. Fereidouni, and M. Osanloo, "Nanoformulating Cinnamomum zeylanicum essential oil with an extreme effect on Leishmania tropica and Leishmania major," *Journal of Drug Delivery Science and Technology*, vol. 63, Article ID 102436, 2021.

[26] M. Reza Mozafari, C. Johnson, S. Hatziantoniou, and C. Demetzos, "Nanoliposomes and their applications in food nanotechnology," *Journal of Liposome Research*, vol. 18, no. 4, pp. 309–327, 2008.

[27] S. Hallaj-Nezhadi and M. Hassan, "Nanoliposome-based antibacterial drug delivery," *Drug Delivery*, vol. 22, no. 5, pp. 581–589, 2015.

[28] S. Song, X. Liu, J. Jiang, Y. Qian, N. Zhang, and Q. Wu, "Stability of triazophos in self-nanoemulsifying pesticide delivery system," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 350, pp. 57–62, 2009.

[29] H. Alipanah, M. Farjam, E. Zarenezhad, G. Roozitalab, and M. Osanloo, "Chitosan nanoparticles containing limonene and limonene-rich essential oils: potential phytotherapy agents for the treatment of melanoma and breast cancers," *BMC Complementary Medicine and Therapies*, vol. 21, no. 1, p. 186, 2021.

[30] S. G. M. Ong, L. C. Ming, K. S. Lee, and K. H. Yuen, "Influence of the encapsulation efficiency and size of liposome on the oral bioavailability of griseofulvin-loaded liposomes," *Pharmaceutics*, vol. 8, no. 3, 2016.

[31] M. Coimbra, B. Isacchi, L. van Bloois et al., "Improving solubility and chemical stability of natural compounds for medicinal use by incorporation into liposomes," *International Journal of Pharmaceutics*, vol. 416, no. 2, pp. 433–442, 2011.

[32] L. Pavoni, R. Pavela, M. Cespi et al., "Green micro- and nanoemulsions for managing parasites, vectors and pests," *Nanomaterials*, vol. 9, no. 9, p. 1285, 2019.

[33] T. Tadros, P. Izquierdo, J. Esquena, and C. Solans, "Formation and stability of nano-emulsions," *Advances in Colloid and Interface Science*, vol. 108, pp. 303–318, 2004.

[34] H. S. Ashbaugh and M. E. Paulaitis, "Effect of solute size and solute–water attractive interactions on hydration water structure around hydrophobic solutes," *Journal of the American Chemical Society*, vol. 123, no. 43, pp. 10721–10728, 2001.

[35] J. Carlsson and J. Åqvist, "Calculations of solute and solvent entropies from molecular dynamics simulations," *Physical Chemistry Chemical Physics*, vol. 8, no. 46, pp. 5385–5395, 2006.

[36] N. Anton and T. F. Vandamme, "Nano-emulsions and micro-emulsions: clarifications of the critical differences," *Pharmaceutical Research*, vol. 28, no. 5, pp. 978–985, 2011.

[37] E. Osman Mohamed Ali, N. A. Shakil, V. S. Rana et al., "Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, rhizoctonia solani and sclerotium rolfsii," *Industrial Crops and Products*, vol. 108, pp. 379–387, 2017.

[38] R. Liang, S. Xu, C. F. Shoemaker, Y. Li, F. Zhong, and Q. Huang, "Physical and antimicrobial properties of peppermint oil nanoemulsions," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 30, pp. 7548–7555, 2012.

[39] A. Sanei-Dehkordi, R. Heiran, M. D. Moemenbellah-Fard, S. Sayah, and M. Osanloo, "Nanoliposomes containing carvacrol and carvacrol-rich essential oils as effective mosquitoes larvicides," *BioNanoScience*, vol. 12, no. 2, pp. 359–369, 2022.

[40] E. Zarenezhad, N. Ranjbar, S. Firooziyan, M. Ghoorkhanian, and M. Osanloo, "Promising larvicidal effects of chitosan nanoparticles containing laurus nobilis and trachyspermum ammi essential oils against *Anopheles stephensi*," *International Journal of Tropical Insect Science*, vol. 42, no. 1, pp. 895–904, 2021.