Nanoemulsion and nanogel containing Artemisia dracunculus essential oil; larvicidal effect and antibacterial activity

Mahmoud Osanloo1*, Samira Firooziyanan, Abbas Abdollahi3, Shekoufeh Hatami4, Amene Nematollahi5, Narges Elahi6 and Elham Zarenezhad7

Abstract
Objective: Microbial infections and mosquito-borne diseases such as malaria, with 627 k deaths in 2020, are still major public health challenges.

Results: This study prepared nanoemulsion and nanogel containing Artemisia dracunculus essential oil. ATR-FTIR analysis (Attenuated Total Reflection-Fourier Transform InfraRed) confirmed the successful loading of the essential oil in nanoemulsion and nanogel. LC50 values (Lethal Concentration 50%) of nanogel and nanoemulsion against Anopheles stephensi larvae were obtained as 6.68 (2–19 µg/mL) and 13.53 (7–25 µg/mL). Besides, the growth of Staphylococcus aureus after treatment with 5000 μg/mL nanogel and nanoemulsion was reduced by ~ 70%. However, about 20% growth of Pseudomonas aeruginosa was reduced at this dose. Considering the proper efficacy of the nanogel as a larvicide and proper antibacterial effect against S. aureus, it could be considered for further investigations against other mosquitoes’ larvae and gram-positive bacteria.

Keywords: Nanotechnology, Infection diseases, Vector-borne disease, Malaria, Anopheles stephensi

Introduction
Malaria is preventable, but it is still the most dreadful vector-borne disease; according to the latest report of WHO, there were about 241 million cases and 627,000 deaths worldwide only in 2020 [1]. Anopheles stephensi Liston is one of the most important malaria vectors in the Middle East and South Asia [2, 3]. Besides, larviciding in 55 countries is one of the most important malaria control methods [1]. However, excessive chemical larvicides have threatened human and environmental health and caused resistance in vectors [4].

Moreover, microbial infections are another health challenge. Staphylococcus aureus (gram-positive) and Pseudomonas aeruginosa (gram-negative) are two common opportunistic bacteria that cause several infections like skin maladies such as pain, swelling, and skin color in humans [5, 6]. Microbial drug resistance and side effects of chemical drugs are other new emerging challenges of the health systems [7, 8]. Therefore, developing new drugs and insecticides with fewer side effects is critical.

For thousands of years, plant-derived extracts and essential oils (EOs) have been widely used as insecticides and natural antibiotics [9, 10]. Moreover, the efficacy of some of them is promising, e.g., Artemisia dracunculus EO with LC50 11.36 µg/mL against A. stephensi [11]. Therefore, this EO was classified in the active category that can be a good alternative to synthetic larvicides [12]. Furthermore, A. dracunculus EO also possesses
and compared to its nanoemulsion. Moreover, their anti-
A. dracunculus
EO were investigated against A. stephensi
first time, the larvicidal effects of a nanogel (containing
A. dracunculus
EO) were investigated against A. stephensi
and compared to its nanoemulsion. Moreover, their anti-
bacterial effects were investigated against S. aureus and P.
aeruginosa.

Main text
Methods and materials
Preparation and characterization of the nanoemulsion
and nanoemulsion-based nanogel
Bark-extracted A. dracunculus EO was purchased from
Zardband Pharmaceuticals Company (Iran). The nanoe-
mulsion was prepared as follows; the EO (100 μL) and
tween 20 (300 μL) was first mixed at 2000 rpm for 3 min
at ambient temperature to form a homogeneous solu-
tion. Distilled water was then added to the mixture up
to desired volume (5000 μL) and was stirred for another
40 min at 2000 rpm. Finally, the prepared nanoeulsion's
droplet sizes and droplet size distribution were investi-
gated utilizing a Dynamic Light Scattering (DLS) appar-
tus (K-One NANO- Ltd. Korea). Droplet size distribution
was computed as d90-d10/d50, where d10, 50, and 90
are percentiles of droplets with diameters less than these
values.

The nanoeulsion was gelified by adding 3.5% w/v car-
boxymethylcellulose; the mixture was stirred at a mild
speed (180 rpm) for 4 h. Moreover, nanoeulsion (-oil)
and nanogel (-oil) were prepared using the same process,
only without the EO.

The viscosity of the prepared nanogel was assayed at
different shear rates at 25 °C under atmospheric pressure
(Rheometer machine model MCR-302, Anton Paar, Aus-
tria). Besides, ATR-FTIR analysis was used to investigate
the successful loading of the EO in the nanoemulsion
and nanogel. Spectra of the EO, nanoeulsion (-oil), nano-
eulsion, nanogel (-oil), and nanogels were recorded in a
wavenumber range of 400–3900 cm⁻¹ using a spectrom-
eter (Tensor II model, Bruker Co, Germany).

Evaluation of larvicidal activity
In the current study, A. stephensi late-3rd or young-4th
instar larvae were used; they were reared and maintained
at 29 ± 2 °C with 70 ± 5% humidity at Urmia University
of Medical Sciences (Iran). Mosquitoes are not exposed
to any insecticides for more than 10 years. According
to the WHO guideline, the larvicidal activity was done
with a slight modification [17]. Briefly, beakers contain-
ing 200 mL of no-chlorine water and 25 larvae were first
prepared. The larvicidal effects of nanoemulsion and
nanogel were then investigated at 6.3, 12.5, 25, 50, and
100 μg/mL. Larval mortality after 24 h exposure was
 counted, while the larvae were not fed during the test.
The larvae were exposed to 1.5 mL ethanol and nanoe-
mulsion (-oil) and nanogel (-oil) in the control and nega-
tive control group.

Evaluation of antibacterial activity
The antibacterial activity of nanoemulsion and nanogel
against S. aureus (ATCC 25,923) and P. aeruginosa (ATCC
27,853) were investigated using ATCC100 standard
method [18]. Briefly, 4 mL of each bacterial suspension
(2 × 10⁵ CFU/mL) was first poured into 5 cm plates sepa-
rate. Antibacterial effects of nanoemulsion and nanogel
were then investigated at 1250, 2500, and 5000 μg/mL.
The treated plates were incubated at 37 °C for 24 h, and
10 μL-microbial suspensions were cultured on agar plates
and incubated for 24 h. The number of grown colonies on
the plates was counted and compared to the control sam-
ples. The control groups were not treated, and the nega-
tive control group was treated with nanoemulsion (-oil)
and nanogel (-oil). Growth (%) of bacteria in each plate
was calculated as (CFU sample /CFU control) × 100.

Statistical analyses
Three replicates were carried out for all tests, and final
values were given as mean ± standard deviations. The
samples were compared with SPSS software using one-
way ANOVA with at least a confidence interval of 95%.

Results
Prepared nanoemulsion and nanogel
DLS profile of the nanoemulsion with a droplet size of
152 ± 6 nm is shown in Fig. 1A. The nanoemulsion had
narrow particle size distribution as its droplet size dis-
bution was 0.98; its single sharp peak also confirmed
its uniform droplet size [19]. The viscosity of nanogel
at different shear rates (1/s) is fully fitted with the Car-
reau-Yasuda models (Fig. 1B). This well-known empirical
equation has been used for non-Newtonian fluids; vis-
cosity is decreased with increasing shear rates [20].

Besides, successful loading of the EO in nanoemulsion
and nanogel was confirmed using ATR-FTIR analysis
(Fig. 1C). The spectrum of A. dracunculus EO showed the
bands at 3061 and 2834 cm⁻¹ displayed –CH
stretching vibration in SP¹ and SP². Besides, the bands
at 1727 and 1638 cm⁻¹ can be related to the stretching
vibration carbonyl C = O group. The peak at 1243 cm⁻¹
corresponds to the stretching vibrations of C-O. The peak
at 1035 cm⁻¹ is assigned to the stretching vibration of C-H. The peak
at 1035 cm⁻¹ is assigned to C-H bending absorption, and
the peak at 808 cm\(^{-1}\) is attributed to benzene rings C-H vibration absorption.

The spectrum of nanoemulsion (-oil) showed broadband between 3300 to 3600 cm\(^{-1}\) can be attributed to the presence of hydroxyl group due to hydrogen bonding. Besides, the peak at 2923 cm\(^{-1}\) is attributed to C-H stretching in tween. Moreover, the peak at 1732 cm\(^{-1}\) corresponds to C=O stretching exhibiting ester groups in tween 20. The sharp band at 1088 cm\(^{-1}\) is assigned to C-O stretching vibration.

In the spectrum of nanogel, the broadband at about 3200 to 3600 cm\(^{-1}\) are related to the hydroxyl group due to hydrogen bonding. The absorbance band at 2923 cm\(^{-1}\) showed CH stretching vibration in tween 20 and EO. Besides, the band at 1734 cm\(^{-1}\) can be related to the carbonyl group in the EO and tween 20. The band at 1457 cm\(^{-1}\) is related to CH\(_2\) bending in the EO and tween 20. All the other characteristic bands appear in the spectra of the EO and nanoeulsion (-oil).

The spectrum of nanogel (-oil) showed the broadband at about 3200 to 3600 cm\(^{-1}\) is attributed to the OH group due to hydrogen bonding. The absorbance band at 2923 cm\(^{-1}\) showed CH stretching vibration in tween 20. The band at 1457 cm\(^{-1}\) is related to CH\(_2\) bending in the EO and tween 20. All the other characteristic bands appear in the spectra of the EO and nanoemulsion (-oil).

The characteristic band at 1579 cm\(^{-1}\) is attributed to the carboxyl group in CMC and tween 20. The characteristic band at 1579 cm\(^{-1}\) is attributed to the carboxyl group in CMC. In the spectrum of nanogel, the broadband at around 3200 to 3600 cm\(^{-1}\) is attributed to the OH group due to hydrogen bonding. The interaction between CMC and the EO during gel formation is related to the preparation of hydrogen bonding. The formation of hydrogen bonds increases the degree of polarization of chemical bonds. Besides, the peak at 1733 cm\(^{-1}\) exhibited carbonyl stretching that confirmed the carbonyl group in CMC, tween 20, and the EO. The peak at 1579 cm\(^{-1}\) corresponded to the carboxyl group in CMC. All the other characteristic peaks appear in the EO and nanogel (-oil) spectra at the same wavenumber.

**Larvicidal effect of the nanoemulsion and nanogel**

Larvicidal effects of nanoemulsion and nanogel against *A. stephensi* are given in Fig. 2. Dose-dependent responses are observed in their efficacy; however, the nanogel with LC50 6.6 (2–19) µg/mL was more potent than the nanoemulsion with LC50 13.5 (7–25) µg/mL. Besides, nanogel was significantly more potent than nanoemulsion at 6.3 µg/mL (P < 0.001), 12.5 µg/mL (P < 0.001), and 50 µg/
mL (P < 0.028). Interestingly, perfect efficacy (100% larval mortality) was observed at 25, 50, and 100 µg/mL nanogel. Moreover, nanoemulsion (-oil) and nanogel (-oil) with 0 and 3% larval mortality did not affect larvae.

Antibacterial effects of the nanoemulsion and nanogel
The antibacterial effect of nanoemulsion and nanogel against *P. aeruginosa* and *S. aureus* are shown in Fig. 3(A and B). The efficacy of nanogel was more potent than nanoemulsion; however, this difference was not significant at all examined concentrations (P > 0.05). The best efficacy (~20% growth inhibitory) against *P. aeruginosa* was observed at 5000 µg/mL nanogel and nanoemulsion. However, 70% growth inhibitory was observed at this point against *S. aureus*. Moreover, nanoemulsion (-oil) and nanogel (-oil) did not affect bacterial growth.

Discussions
The preparation of nanostructures-loaded EOs has received more attention as a promising approach to developing new natural insecticides and drugs [15]. Efficacy of such mentioned nanosystems against important mosquitoes’ larvae, including *Aedes* (spp.), *Anopheles* (spp.), and *Culex* (spp.), have been reported in the literature. For instance, LC50 of *Lippia alba* EO nanoemulsion against *A. aegypti* was 31.02 µg/mL [21]. LC50 value of nanoemulsion of *Mentha piperita* EO against *C. Pipiens* was 31.24 µg/mL [22]. Besides, nanocrystal emulsion of *Ficus glomerata* EO with LC50 17 µg/mL against *A. stephensi* showed proper efficacy [23]. The current study investigated the larvicidal effect of nanogel containing *A. dracunculus* EO for the first time against *A. stephensi*. Its efficacy was more potent than nanoemulsion due to its proper stability and sustained release profile. Nanogels with soft tissue, high drug loading capacity, biocompatibility, biodegradability, good swelling ability, and structural stability have recently received more attention [24–26].

Bacterial infections may cause serious diseases in humans and animals [27, 28]. In the current research, the efficacy of both nanoemulsion and nanogel against *S. aureus* (gram-positive) was more than *P. aeruginosa* (gram-negative). This agrees with the literature; gram-negative bacteria with an extra outer membrane are more resistant to antibiotics than Gram-positive bacteria [29]. However, the Gram-positive bacteria cell wall structure allows hydrophobic molecules to penetrate the cells easily [30].

Some reports on the antibacterial effects of nanoemulsion and nanogel containing EOs have been found in the literature. For instance, thyme EO nanoemulsion reduced *E. coli* populations by 3.28–4.13 log CFU/mL [31]. Moreover, the growth of *P. aeruginosa* after treatment with 2500 and 5000 µg/mL of nanogel containing *Mentha longifolia* EO was reduced by 5 and 90%. On the other hand, the growth of *S. aureus* after treatment with such doses was reduced by 3 and 100% [6].
Conclusions
A comprehensive comparison was carried out on the efficacy of nanoemulsion and nanogel containing *A. dracunculus* EO. The nanogel at 25, 50, and 100 µg/mL concentrations showed perfect larvicidal effects on *A. stephensi*. Moreover, the antibacterial properties of the nanoemulsion and nanogel were equal to each other and showed better efficacy against *S. aureus* than *P. aeruginosa*.

Limitations
The efficacy of the nanoemulsion and nanogel could be investigated against other important mosquitoes’ larvae. In addition, it is recommended to investigate the efficacy of the nanoemulsion and nanogel on clinical isolated bacteria strains.

Fig. 3 Antibacterial effects of nanoemulsion and nanogel containing *A. dracunculus* EO against *A. P. aeruginosa* and *B. S. aureus*.

Abbreviations
EO: Essential Oil; ATR-FTIR: Attenuated Total Reflection-Fourier Transform Infra-Red; DLS: Dynamic Light Scattering; LC50: Lethal Concentration 50.

Acknowledgements
Not applicable.

Author contributions
MO designed the study and analyzed the data. MO and ShH drafted the MS. SF performed larvicidal bioassays. AA performed antibacterial tests. AN reviewed the literature. NE prepared the nanoformulations. EZ interpreted ATR-FTIR spectra. All authors read and approved the final manuscript.

Funding
Fasa University of Medical Sciences funded this study, grant No. 400175.

Availability of data and materials
All data generated or analyzed during this study are available from the corresponding author on reasonable request.
Declarations

Ethics approval and consent to participate
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.1400.164. Moreover, all methods in the current study were performed according to the WHO (World Health Organization) relevant guidelines and national regulations.

Consent for publication
Not applicable.

Competing interests
Researchers have no conflict of interest in this study.

Author details
1. Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fasa, Iran. 2. Medical Entomology, Disease Control Unit, Urmia Health Center, Urmia University of Medical Sciences, Urmia, Iran. 3. Department of Microbiology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran. 4. Department of Chemistry, School of Medicine, Fasa University of Medical Science, Fasa, Iran. 5. Department of Food Safety and Hygiene, School of Health, Fasa University of Medical Sciences, Fasa, Iran. 6. Department of Tissue Engineering, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fasa, Iran. 7. Non-communicable Disease Research Center, Fasa University of Medical Sciences, Fasa, Iran.

Received: 16 April 2022   Accepted: 21 June 2022
Published online: 12 August 2022

References
1. Organization WH. World malaria report 2021. 2021.
2. Surendran SN, Sivabalakrishnan K, Sivasingingam A, Jayadas TT, Karuvannak K, Santhirasegaran S, Gajapathy K, Senthilnathanman M, Karunaratne S, Ramasamy R. Anthropogenic factors driving recent range expansion of the malaria vector Anopheles stephensi. Front Public Health. 2019;7:53.
3. Sharma A, Deeshmukh A, Sharma R, Kumar A, Mukherjee S, Chandra G, Gokhri S. Population genetic structure of malaria vector Anopheles stephensi using mitochondrial Cytochrome oxidase II gene in Indian populations. Indian J Exp Biol. 2011;49(10):996–1002.
4. Vatandoost H, Hanafi-Bojd AA, Nikipoor F, Raesi A, Abai MR, Zaim M. Situation of insecticide resistance in malaria vectors in the World Health Organization of Eastern Mediterranean region 1990–2020. Toxicol Res. 2022;11:1.
5. Abdollahi A, Zarenehzad E, Osanloo M, Ghaznavi G, Khalili Pour M. Promising antibacterial activity of a mat of polycaprolactone nanofibers impregnated with a green nanogel. Nanomed Res J. 2020;5(2):192–201.
6. Qasemi H, Fereidouni Z, Karimi J, Abdollahi A, Zarenezhad E, Rasti F, Osanloo M. Promising antibacterial effect of impregnated nanofiber mats with a green nanogel against major human pathogens. BioNanoScience. 2021;11(2):549–58.
7. Abedinpour N, Ghanbariasad A, Taghinezhad E, Osanloo M. High antibacterial effect of impregnated nanofiber mats with a green nanogel against major human pathogens. BioNanoScience. 2021;11(2):428–36.
8. Avazmohammadi R, Castañeda PP. The rheology of non-dilute dispersions of highly deformable viscoelastic particles in Newtonian fluids. J Fluid Mech. 2015;763:386–432.
9. Ferreira RM, Duarte JL, Cruz RA, Oliveira AE, Araujo RS, Carvalho JC, Mourao RH, Souito RN, Fernandes CP. A herbal oil in water nano-emulsion prepared through an ecofriendly approach affects two tropical disease vectors. Neovirus. 2019;29(6):778–84.
10. Esmali F, Sanei-Dehkordi A, Amoozegar F, Osanloo M. A review on the use of essential oil-based nanoformulations in control of mosquitoes. Biointerpharm. 2022;17:168–76.
11. WHO:WHO. Guidelines for laboratory and field testing of mosquito larvicides. In: 2005.
12. Abdollahi A, Mirzaei E, Amoozegar F, Moemenbollah-Fard MD, Zarenehzad E, Osanloo M. High antibacterial effect of impregnated nanofiber mats with a green nanogel against major human pathogens. BioNanoScience. 2021;11(2):549–58.
13. Ebadi M, Tajik H, Razavi RS, Mahram M, Moradi M, Hajimohammadi B, Naghili H, Hashemi M, Mehdi-zadeh T. Essential oil of tarragon (Artemisia dracunculus) antibacterial activity on Staphylococcus aureus and Escherichia coli in culture media and Iranian white cheese. Iran J Microbiol. 2012;4(1):30.
14. Yousefpoor Y, Amani A, Divsalar A, Mousavi SE, Shakeri A, Sabzevari JT. Anthro- rheumatic activity of topical nanoemulsion containing bee venom in rats. Eur J Pharm Biopharm. 2022;172:168–76.
15. Esmali F, Sanei-Dehkordi A, Amoozegar F, Osanloo M. A review on the use of essential oil-based nanoformulations in control of mosquitoes. Biointerpharm. 2022;11(5):12516–29.
16. Ghiyanni N, Aghazadeh H, Raeesian H, Zadeh S, Osanloo M. Preparation of nanoparticles of mentha piperita essential oil and investigation of their cytotoxic effect on human breast cancer lines. Biointerpharm. 2021;11(2):428–36.
17. Avazmohammadi R, Castañeda PP. The rheology of non-dilute dispersions of highly deformable viscoelastic particles in Newtonian fluids. J Fluid Mech. 2015;763:386–432.
18. Ferreira RM, Duarte JL, Cruz RA, Oliveira AE, Araujo RS, Carvalho JC, Mourao RH, Souito RN, Fernandes CP. A herbal oil in water nano-emulsion prepared through an ecofriendly approach affects two tropical disease vectors. Neovirus. 2019;29(6):778–84.
19. Jesser EN, Yegueuman CO, Gillo VJ, Santillan GO, Murray AP, Domini AP, Verdin Gonzalez JO. Optimization and characterization of essential oil nanoemulsions using ultrasound for new ecofriendly insecticides. ACS Sustain Chem Eng. 2020;8(21):7981–92.
20. Nazeri AA, Rajan HV, Vijayakumar SD, Saravanak M. Evaluation of larvicidal and repellent activity of nanocrystal emulsion synthesized from Flugomerata and neem oil against mosquitoes. JClust Sci. 2019;30(6):1649–61.
21. Cuggino JC, Blanco ERO, Gugliotta LM, Iqbalzada QA, Calderon M. Crossing biological barriers with nanogels to improve drug delivery performance. J Control Release. 2019;307:221–46.
22. Pinelli F, Perale G, Rossi F. Coating and functionalization strategies for nano- gels and nanoparticles for selective drug delivery. Gels. 2020;6(1):16.
23. Malm E, Giannitelli SM, Trombetta M, Rainer A. Synthesis of nanogels: cur- rent trends and future outlook. Gels. 2021;7(2):36.
24. Salem ME, Elansary HO, Al-HM, B-Settawy AA, Eshkhs MS, Abdel-Salam EM, Skalicka-Woźniak K. Bioactivity of essential oils extracted from Cypres suis macropodan branchlets and Cymusites citrodora leaves grown in Egypt. BMC Complement Altern Med. 2018;18(1):1–7.
25. Elansary HO, Scopa A, Kubica P, Ertel H, Al-HM, Eshkhs MS, Abdel-Salam EM, El-Esawi M, El-Ansary DO. Bioactivities of traditional medicinal plants in Alexandria. Evid Based Complement Alternat Med. 2018;2018:1.
26. Trombeta D, Castelli F, Sarpietro MG, Venuti V, Cristiani M, Daniele C, Saija A, Mazzanti G, Bisignano G. Mechanisms of antibacterial action of three monoterpene: Antimicrobial Agents Chemother. 2005;49(6):2474–89.
27. Puri VV, Lakshmikantham K, Bourke P, Cullen P. Application of natural antimicrobials for food preservation. J Agric Food Chem. 2009;57(14):5987–6000.
28. Guo M, Zhang L, He Q, Arabi SA, Zhao H, Chen W, Ye X, Liu D. Synergistic antibacterial effects of ultrasound and thyme essential oils nanoemulsion against Escherichia coli O157:H7. Ultrason Sonochem. 2020;66. 104988.
