

Original Article
Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org



doi: 10.4103/1995-7645.271292

Impact factor: 1.77

Nanoemulsified *Mentha piperita* and *Eucalyptus globulus* oils exhibit enhanced repellent activities against *Anopheles stephensi*

Rahman Mohammadi¹, Mehdi Khoobdel^{1✉}, Maryam Negahban², Solmaz Khani³

¹Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

²Iranian Research Institute of plant protection, Tehran, Iran

³Faculty of Agriculture, Department of Plant Protection, University of Tabriz, Tabriz, Iran

ARTICLE INFO

Article history:

Received 4 May 2019

Revised 19 September 2019

Accepted 23 September 2019

Available online 26 November 2019

Keywords:

Essential oil

N,N-diethyl-m toluamide

Nanoemulsion

Eucalyptus

Mentha

Mosquito repellent

ABSTRACT

Objective: To formulate nanoemulsion from essential oils of *Mentha (M.) piperita* L. and *Eucalyptus (E.) globulus* L. and to compare their repellent activity with normal essential oils and N,N-diethyl-m toluamide (DEET) as a standard chemical compound.

Methods: In this study, protection time of essential oils and DEET was evaluated on four human subjects using test cage, and their values were determined against *Anopheles stephensi*. Furthermore, ED₅₀ values for the above essential oils were determined using the ASTM E951-94 method. The compositions of essential oils were determined using GC-MS, and droplet size and zeta potential of the nanoemulsion were measured with dynamic light scattering.

Results: The results (expressed as mean±SD) showed that protection time of *M. piperita* 50%, *M. piperita* Nano 50%, *E. globulus* 50%, *E. globulus* Nano 50%, and DEET 25% was (2.89±0.45) h, (4.17±0.28) h, (0.96±0.27) h, (5.51±0.02) h, and (6.10±0.47) h, respectively. ED₅₀ values were 29.10 (95% CI: 23.36-36.06) µg/cm² for *Mentha*, 19.39 (15.35-23.99) µg/cm² for *Mentha* Nano, 36.10 (28.70-48.01) µg/cm² for *Eucalyptus*, 18.50 (14.65-23.23) µg/cm² for *Eucalyptus* Nano, and 3.62 (2.68-4.55) µg/cm² for DEET, respectively. *E. globulus* Nano and *M. piperita* Nano provided significantly longer protection than normal essential oils *E. globulus* and *M. piperita* (P<0.01).

Conclusions: The preparation of nanoemulsion from the essential oils of *M. piperita* and *E. globulus*, significantly increases the protection time and reduces ED₅₀ values of these essential oils, hence, *M. piperita* Nano and *E. globulus* Nano can be good alternatives to DEET and other chemical compounds.

1. Introduction

About 36% of the world's population, mostly children under the age of five years, is affected by malaria[1,2]. There are about 447 860 fatal cases every year because of malaria, which is one of the deadliest mosquito-borne diseases[3]. *Anopheles (An.) stephensi* mosquitoes are recognized as the most important cause of malaria

in central and southern Asia[4]. Continuous use of chemicals to control malaria-transmitting mosquitoes causes resistance in these mosquitoes, and also contaminates the environment[5,6]. One of the widely used insect repellents is the chemical compound N,N-diethyl-m toluamide (DEET), which is considered as a "gold

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2019 Asian Pacific Journal of Tropical Medicine Produced by Wolters Kluwer- Medknow. All rights reserved.

How to cite this article: Mohammadi R, Khoobdel M, Negahban M, Khani S. Nanoemulsified *Mentha piperita* and *Eucalyptus globulus* oils exhibit enhanced repellent activities against *Anopheles stephensi*. Asian Pac J Trop Med 2019; 12(11): 520-527.

✉Corresponding author: Mehdi Khoobdel, Health Research Center, Baqiyatallah University of Medical sciences, Tehran, Iran.
Tel: 0989126180432
Fax: 0989126180432
E-mail: khoobdel@yahoo.com

standard” in the experiments due to its long-term provision of protection[7]. Various studies on DEET have shown that DEET is highly detrimental (including skin toxicity, seizure, or acute manic syndrome), causing equipment defects, including glasses and cell phones, as well as dissatisfaction among consumers[8]. Therefore, various researchers are trying to reduce such complications and improve the effectiveness of different and safe materials, thereby, providing more satisfaction among consumers.

Essential oils of many herbs extracted from different plant parts (flowers, tubers, leaves, fruits, branches, and roots) contain compounds with toxic, insect-repellant properties[9]. Results obtained from various studies on mint (*Teucrium leucocladum*), citronella (*Cymbopogon nardus*), basil (genus *Ocimum* and their cultivars), thyme (*Thymus vulgaris* L.), neem (*Azadirachta indica* A. Juss), and lemongrass (*Cymbopogon citratus*) have shown their repellent properties on various insects, especially mosquitoes[10-12]. It is normally clear that the repellent effects of herbal essential oils are less than those of such synthetic substances as DEET, but their effectiveness can be increased by modification of essential oils or combination of several essential oils[13,14].

To maintain the biological activity of essential oils and prevent the evaporation of their constituents, it is necessary to formulate essential oils[15,16]. Nanoformulation of herbal essential oils leads to a small droplet size, a high physical stability, and high bioavailability of the emulsion[17,18], with sizes often ranging from 20 nm to 500 nm according to different criteria of researchers[19]. Different studies have shown that nanoemulsion based on herbal essential oils has a high potential for controlling mosquitoes[8,20,21]. Therefore, this study aimed to provide nanoemulsion from the essential oils of *Mentha (M.) piperita* L. and *Eucalyptus (E.) globulus* L., and to compare the repellency effects of these nanoemulsion compounds with normal essential oils and DEET against *An. stephensi*.

2. Materials and methods

2.1. Materials

Essential oils were prepared from aerial parts of *M. piperita* L. (Batch Number: 002-361-18), and from the leaves of *E. globulus* L. (Batch Number: 001-184-34) obtained from the Zardband Medicinal Plants Inc. (Iran). DEET with CAS NUMBER 3-62-134 and a density of 0.99 g/mL, ethanol, polysorbate 80, polyethylene glycol, and butanol were procured from Merck Chemicals Inc. (Germany). Mature female mosquitoes were obtained from the insectarium of mosquito nursery (Baqiyatallah University of Medical Sciences) where they have been raised at (27±3)°C, a relative humidity of (80±10)%, and a photoperiod of 16 h light: 8 h dark.

2.2. GC–MS analysis

In this study, essential oils of *M. piperita* L. and *E. globulus* L. were analyzed by GC-MS (Agilent 6890N) coupled with a mass spectrometer (Agilent 5973) with a strong library for identification of isolated substances, located at the Faculty of Chemistry, Tabriz University. The gas chromatograph is equipped with a hairpin column. The mass range is detectable in the range of 2-800 amu. Helium gas (He 99.99%) was used as the carrier gas. The ionization system of the MS is an electron ionization type with a relatively high electron energy of 70 electron volts, and the use of this system leads to direct ionization of molecules. The filter used in this system is quadrupole. Only the masses that match the parameters of this filter pass through the filter at a specific time and arrive at the detector. Column specifications of this GC-MS: HP 5973, Capillary Column, Model Number: Agilent 19091S-433, HP-5MS, 0.25 mm×30 m×0.25 µm, Max temperature: 350 °C, Nominal length: 30.0 m, Nominal diameter: 250.00 µm, Nominal film thickness: 0.25 µm, Mode: constant flow, Initial flow: 1.0 mL/min, Nominal init pressure: 8.76 psi, Average velocity: 37 cm/sec, Inlet: Front Inlet, Outlet: MSD, Outlet pressure: vacuum.

2.3. Preparation of nanoemulsion 50% from essential oils of mint and eucalyptus

First, 13 mL (21%) of polyethylene glycol (Bipolar and Emulsifier) was poured into a beaker and homogenized by a homogenizer (MICCRA D9 45043) at 11 000 rpm. Then, 10 mL (16%) of polysorbate 80 (Bipolar and Emulsifier) was instilled into polyethylene glycol under the homogenizer to dissolve well at a speed of 11 000 rpm for 5 min. Next, 5 mL (8%) of *Sesamum indicum* L. oil (carrier and synergist oil) was added. The synergist oil was instilled on the two previous substances and homogenized for proper mixing. Then, 30 mL (50%) of pure peppermint or eucalyptus essential oil was added and the mixture left until these ingredients were combined well under the homogenizer. After about 10 min, 2 mL (5%) of butanol (Bipolar and Emulsifier) was added due to its bipolarity and better dissolution of the compounds, and then placed under a homogenizer at 11 000 rpm and 25 °C for 5 min.

2.4. Measuring droplet size and zeta potential of the nanoemulsion

Droplet size and zeta potential of the nanoemulsion were measured by a dynamic light scattering device of Nanotrak Wave model (Microtrak Inc.) with a measurement range of (8-6 500) nm, and a zeta potential measurement range of (20-200) mV in the Central Laboratory of Tabriz University.

2.5. Repellency tests

This research employed four male volunteers aged 26–32 years (29 years on average) to determine the protection time, failure time, and effective doses. The volunteers were recommended to avoid the use of perfume, colognes, chewing gum, cigarettes, caffeinated materials (e.g. tea and coffee) as well as hair gel, fragrant soap, and redolent chocolate 12 h prior to and during the test^[22,23].

It should be noted that before the test, informed consent was obtained from all volunteers. This research was approved by the Ethics Committee of Baqiyato-Allah University of Medical Sciences, Tehran, Iran (Approval ID: IR. Bmsu. REC. 1396.202).

2.5.1. Skin allergy test of human volunteers

The volunteers' arms were first disinfected using 72% alcohol. For skin allergy test, a circle with a surface area of 6.6 cm² was drawn at the upper arm of each volunteer using a standard model. Subsequently, 50 µL of essential oils and DEET were spread on the drawn circle by a sampler. Then, the test candidate was advised not to contact the test area with water for 2 d^[22]. After 3 d, no symptoms of skin allergy (such as burning, itching, inflammation, and skin redness) were seen in all the volunteers.

2.5.2. Important conditions for mosquitoes used in the repellency test

Adult female, non-blood fed, and nulliparous mosquitoes of 7–8 days old were kept in cages [(50×50×50) cm] and not fed 10–12 h prior to repellency tests by removing 10% sugar solutions from the mosquito cage. Then, in the event of sitting or fleshing the snout or biting without blood feeding, the presence of 10–20 mosquitoes on the forearm of the volunteer for 30 sec showed the suitability of mosquitoes to start the test^[23].

2.5.3. Estimation of protection and failure time

The protection and failure time were estimated for the repellents on essential oils from *M. piperita* 50%, *M. piperita* Nano 50%, *E. globulus* 50%, *E. globulus* Nano 50%, and also for DEET 25%. As solvents, absolute ethanol alcohol was used for non-formulated essential oils and DEET, and distilled water for nanoemulsion oils. The volunteer's hands were then impregnated with the repellents (1.5 mL–2.0 mL) from the elbows to the wrists by a sampler. The volunteer's hand was covered by latex gloves to prevent mosquitoes from biting in the area below the wrists and fingers not impregnated with the repellents. After 5 min of hand impregnation with the repellents, the volunteer placed his forearm in a cage containing about 200 blood-deprived mosquitoes for 3 min. Any biting, probing,

and sitting of mosquitoes on the skin were recorded during the above 3 min. Thereafter, the volunteers were kept without any activity and contact of impregnated parts with various surfaces for 30 min. The 3-min test and 30-min rest periods continued until two bites occurred in a 3-min test or two bites in two common 3-min tests at 30 min intervals. If the bite was not confirmed within subsequent 3 min after a bite in a 3-min test, the test continued until a bite was confirmed. To determine the failure times of the substances, the test confirmation did not stop after receiving a bite and continued until the 10th bite. After each test, the mosquitoes used in the previous test were discarded and not used for subsequent tests. To determine the protection time and failure time, each repellent was used for four volunteers with three replications^[22,23].

2.5.4. Estimation of effective doses

The effective doses of repellents were estimated through ASTM E951-94 method using a Plexiglas kit with dimensions of (22×5) cm and five cells measuring (3×4) cm. Of the five cells plotted on the volunteer's forearm, four cells were meant for preparation of serial concentrations of repellents based on absolute ethanol or distilled water made from the repellents. A final cell was considered as a control (pure ethanol or distilled water). The control cell was impregnated (by a 45 µL sampler) with distilled water as a control for nanoemulsion essential oils, and pure ethanol as a control for common essential oils and DEET. Each of the other four cells received successively 45 µL of a repellent of various concentrations. After impregnation, there was a 5-min rest, then the ASTM test cage was closed on the volunteer's hand. Under each five cells, there was a sliding drawer that opened and closed. In each cell, five mature female mosquitoes aged 7–8 days, which were not blood fed and kept hungry for 12 h, were introduced using an aspirator. By pulling the drawer, the mosquitoes were simultaneously in contact with the skin impregnated with different concentrations of repellents. The number of bites was recorded within 5 min of contact and every 5 min accounted for a test.

2.5.5. Statistical analysis

To determine the effective dose of each repellent material, four volunteers were used with three replications. After recording the results, ED₅₀ and ED₉₀ values in the tested compounds were estimated by probit regression analysis in SPSS 21 software. Dose-effect lines of these compounds were also drawn with Excel software.

Values of protection and failure time were expressed as mean±standard deviation (SD); Also means of them were compared by the ANOVA, Tukey test. The 1% level was employed in tests of significance.

3. Results

3.1. GC-MS analysis

The GC-MS results of essential oil analysis for *M. piperita* showed that the three main components of this essential oil were D-Limonene (19.72%), thymol (19.02%) and carvacrol (12.37%) (Table 1). The results of GC-MS analysis for the essential oil of *E. globulus* revealed that the main components of this essential oil were 1,8-cineole (59.45%) and terpinene γ- (10.91%) (Table 2). GC/MS profile of essential oil from leaves of *E. globulus* and aerial parts of *M. piperita* are presented in Figure 1A and 1B.

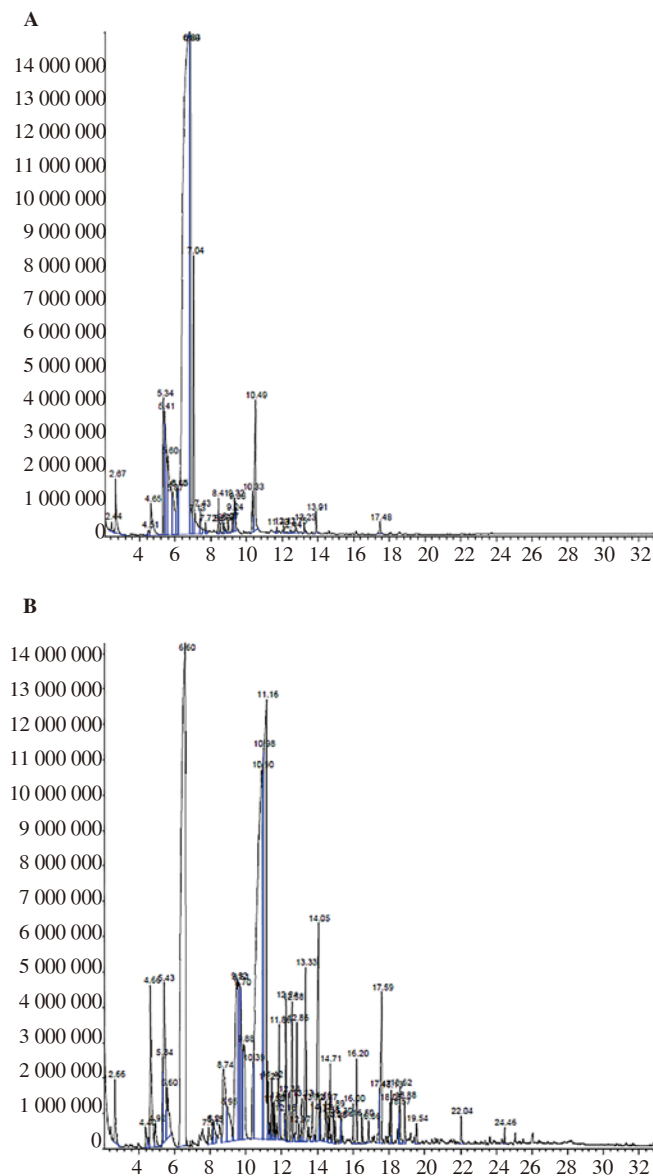


Figure 1. GC/MS profile of essential oil from (A) leaves of *Eucalyptus globulus* L.; (B) aerial parts of *Mentha piperita* L.

Table 1. GC-MS analysis of *Mentha piperita* essential oil.

NO.	RT	Compounds	Compounds %	RI lit
1	2.66	Octane	0.59	800
2	4.40	Pinene α->	2.37	932
4	4.93	Camphene	0.35	943
5	5.33	Sabinene	0.70	969
6	5.43	Pinene β->	2.71	974
7	5.60	beta.-Myrcene	1.00	988
8	6.59	D-Limonene	19.72	1 024
9	7.91	1,8-Cineole	0.32	1 026
10	8.14	Limonene oxide $\langle\text{cis}\rangle$->	0.19	1 132
11	8.21	Limonene oxide $\langle\text{trans}\rangle$->	0.35	1 137
12	8.74	Menthone	2.02	1 153
13	8.95	Borneol	1.19	1 165
14	9.52	Menthol $\langle\text{iso}\rangle$->	4.28	1 179
15	9.62	neo-Menthol	3.05	1 184
16	9.69	Dihydrocarvone, trans-(+)-	2.50	1 200
17	9.87	Neodihydrocarveol	2.14	-
18	10.39	Pulegone	0.77	1 233
19	10.89	Thymol	19.02	1 289
20	10.97	Menthyl acetate	4.29	1 294
21	11.16	Carvacrol	12.37	1 298
22	11.25	Carvone oxide $\langle\text{cis}\rangle$->	0.30	1 259
23	11.41	Carvone oxide $\langle\text{trans}\rangle$->	0.33	1 273
24	11.51	Bornyl acetate	0.23	1 284
25	11.86	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-,cis	0.88	-
26	12.24	Dihydro carveol acetate	1.05	1 306
27	12.38	Carvyl acetate $\langle\text{trans}\rangle$->	0.31	1 339
28	12.58	carveol acetate	1.28	1 356
29	12.86	Carvyl acetate $\langle\text{cis}\rangle$->	0.98	1 365
30	12.97	Piperitenone oxide	0.10	1 366
31	13.14	Bourbonene β->	0.42	1 387
32	13.33	Elemene β->	1.89	1 389
33	13.72	Cis-Jasmone	0.44	1 392
34	14.05	Caryophyllene $\langle\text{Z}\rangle$->	2.85	1 408
35	14.15	Humulene α->	0.22	1 452
36	14.47	Farnesene $\langle\text{E}\rangle$- <math>\beta< math>-><="" td=""><td>0.41</td><td>1 454</td></math>\beta<>	0.41	1 454
37	14.71	Germacrene D	0.61	1 484
38	16.00	Amorphene δ->	0.26	1 511
39	16.20	Calamenene $\langle\text{cis}\rangle$->	0.63	1 528
40	16.50	Cadinene α->	0.18	1 537
41	17.46	Spathulenol	0.55	1 577
42	17.58	Caryophyllene oxide	1.86	1 582
43	18.05	Viridiflorol	0.28	1 592
44	18.13	Naphthalene $\langle\text{2-acetyl}\rangle$->	0.34	1 608
45	18.56	Caryophylla-4(12),8(13)-dien-5-ol	0.23	1 639
46	18.61	Alpha-cadinol	0.47	1 652
47	-	Other compounds	2.97	-
Total			100	

3.2. Droplet size and zeta potential of nanoemulsion

The results obtained from the analysis of droplet size and zeta potential of the nanoemulsion showed a mean droplet size of about 11.32 nm (Figure 2A) with a zeta potential of 9.50 mv for the essential oil of *M. piperita*. Particle size distribution profile of the *M. piperita* Nano and *E. globulus* Nano essential oils are presented in Figure 2A and 2B by number. Droplet size was approximate 103.90 nm (Figure 2B) with a zeta potential of 27.00 mv for *E. globulus* Nano essential oil.

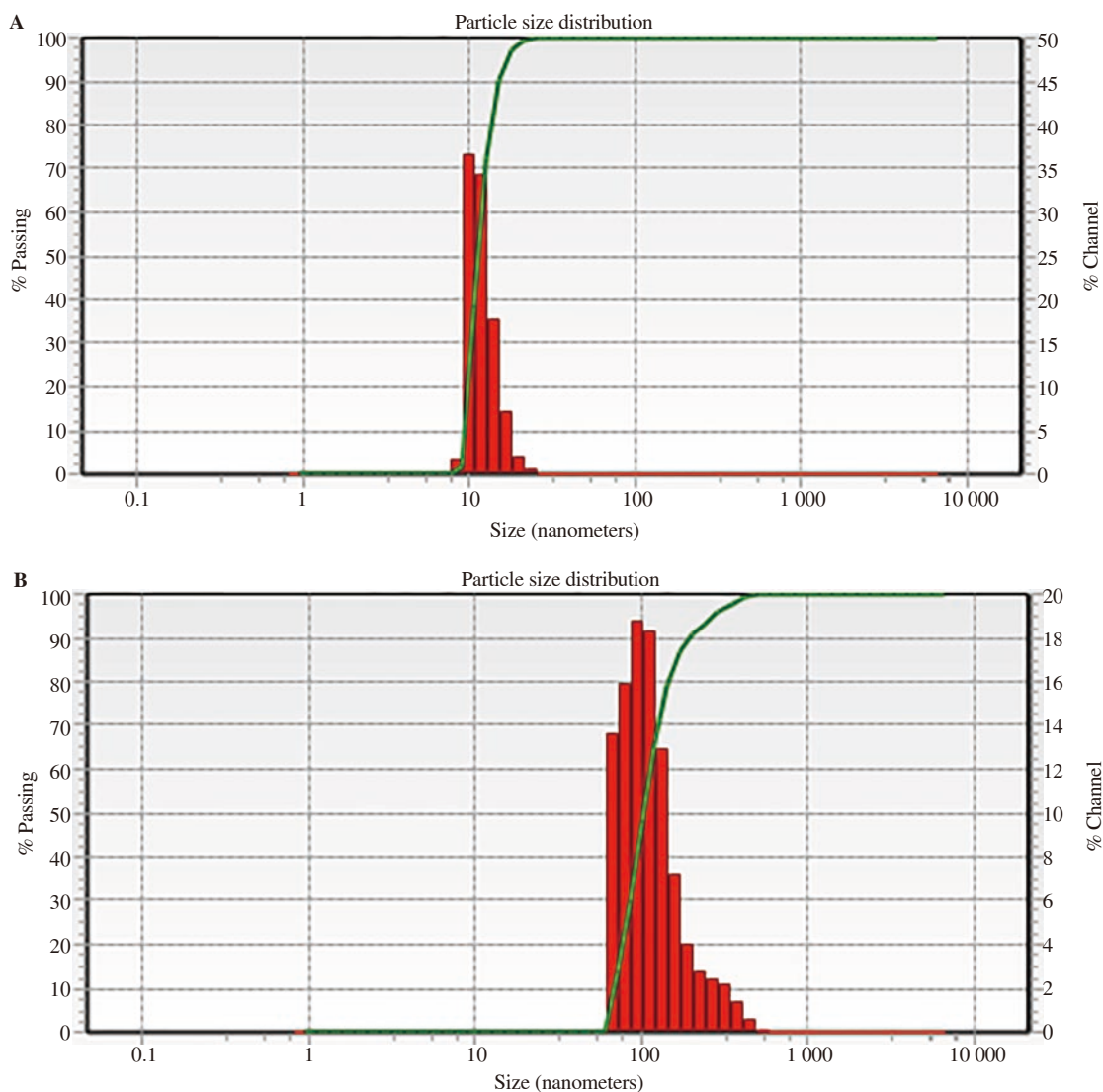


Figure 2. Particle size distribution profile: (A) *Mentha piperita* Nano essential oil; (B) *Eucalyptus globulus* Nano essential oil. Size distribution is presented by number.

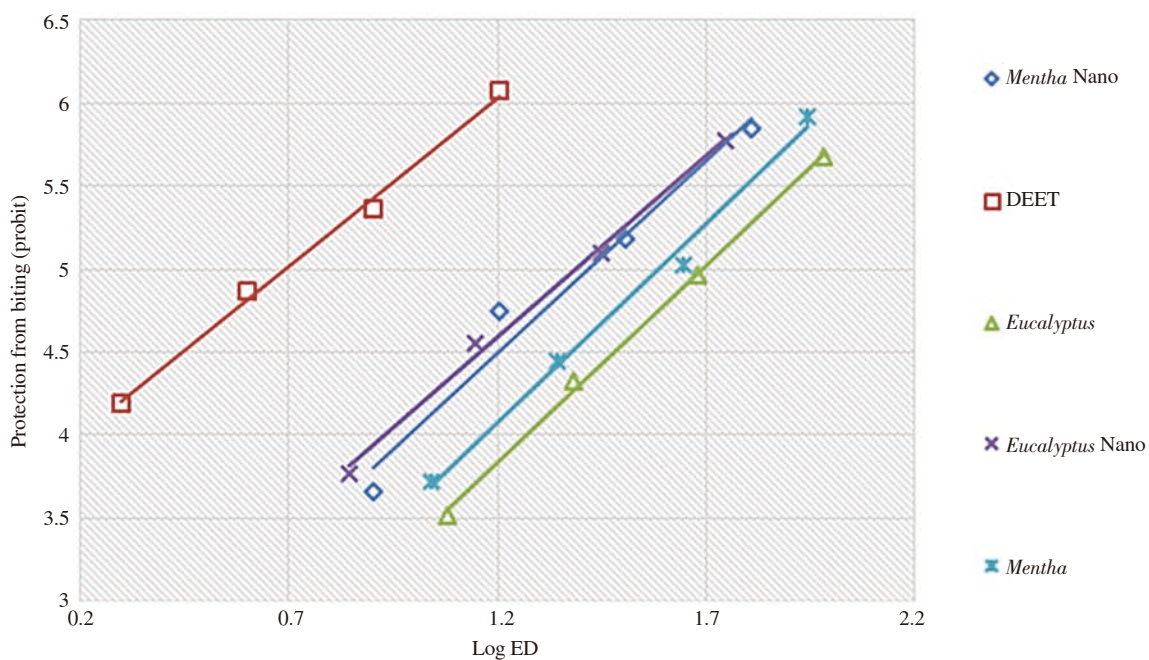


Figure 3. Dose-response line for DEET and normal and nanoemulsion essential oils against *Anopheles stephensi*.

Table 2. GC-MS analysis of *Eucalyptus globulus* essential oil.

NO.	RT	Compounds	Compounds (%)	RI lit
1	2.44	Nonane <n->	0.12	900
2	2.66	Octane	1.02	910
3	4.52	Thujene <α->	0.13	924
4	4.64	Pinene <α->	1.16	932
5	5.34	Sabinene	2.62	969
6	5.41	Sabinene	5.00	969
7	5.60	Pinene <β->	4.42	974
8	5.86	<β->Thujene	1.69	978
9	6.10	Terpinene <α->	0.93	1 014
10	6.80	1,8-Cineole	59.45	1 026
11	6.83	Terpinene <γ->	10.91	1 054
12	7.03	Terpinolene	3.37	1 086
13	7.12	Acetaldehyde	0.87	-
18	8.72	Menthone	0.12	1 148
19	8.97	Borneol	0.17	1 165
20	9.24	alpha-Terpineol	0.25	1 186
21	9.32	Dihydrocarvone<cis->	0.48	1 191
22	10.49	Pulegone	3.07	1 233
23	11.70	Menth-1-en-9-ol <ρ->	0.12	1 294
24	12.75	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis	0.20	-
25	13.22	Bourbonene <β->	0.13	1 387
26	13.90	Caryophyllene <(Z)->	0.30	1 406
27	-	Other compounds	3.47	-
Total			100	

3.3. Protection time

The protection time analysis showed that the essential oils provided protection with an average of (2.89±0.45) h for *M. piperita* 50%, with an average of (4.17±0.28) h for *M. piperita* Nano 50%, with an average of (0.96±0.27) h for *E. globulus* 50%, with an average of (5.51±0.02) h for *E. globulus* Nano 50%, and with an average of (6.10±0.47) h for DEET 25% (Table 3). Comparing the protection time, *E. globulus* Nano and *M. piperita* Nano are significantly higher than protection time for essential oils of *E. globulus* and *M. piperita* (*P* both <0.01). It's also observed that the protection time (mean±SD) of DEET was significant longer compared to *M. piperita*, *M. piperita* Nano, *E. globulus*, and *E. globulus* Nano (*P* all <0.01).

3.4. Failure time

The failure times obtained showed their effectiveness with an average of (3.99±0.45) h for *M. piperita* 50%, with an average of (5.09±0.45) h for *M. piperita* Nano 50%, with an average of (2.06±0.27) h for *E. globulus* 50%, with an average of (6.51±0.02) h for *E. globulus* Nano 50%, and with an average of (7.12±0.47) h for DEET 25% (Table 3). Statistical comparison of the data revealed that failure time for *E. globulus* Nano and *M. piperita* Nano are significantly longer than failure time for essential oils *E. globulus* and *M. piperita* (*P* both <0.01). It's also observed that failure time (mean±SD) of DEET was significant longer compared to *M. piperita*, *M. piperita* Nano, *E. globulus*, and *E. globulus* Nano (*P* all <0.01).

Table 3. Comparison of protection time and failure time for normal and nanoemulsion essential oils 50% with DEET 25% against *Anopheles stephensi* at 1% probability level.

Repellents	Protection time (h)		Failure time (h)	
	Mean±SD	Range	Mean±SD	Range
<i>Mentha piperita</i> 50%	2.89±0.45 ^d	2.25-3.35	3.99±0.45 ^d	3.35-4.45
<i>Mentha piperita</i> Nano 50%	4.17±0.28 ^e	3.90-4.45	5.09±0.45 ^e	4.45-5.55
<i>Eucalyptus globulus</i> 50%	0.96±0.27 ^a	0.60-1.15	2.06±0.27 ^a	1.70-2.25
<i>Eucalyptus globulus</i> Nano 50%	5.51±0.02 ^b	5.50-5.55	6.51±0.02 ^b	6.50-6.55
DEET 25%	6.10±0.47 ^a	5.60-6.60	7.12±0.47 ^a	6.70-7.86

^{abcde}Means followed by different letters are significantly different (*P*<0.01). Based on ANOVA, Tukey's test.

3.5. Effective doses

The results obtained for different compounds showed that ED₅₀ and ED₉₀ values for the essential oils were 29.10 µg/cm² and 139 µg/cm² in *M. piperita*, 19.39 µg/cm² and 94 µg/cm² in *M. piperita* Nano, 36.10 µg/cm² and 199 µg/cm² in *E. globulus*, 18.50 µg/cm² and 98 µg/cm² in *E. globulus* Nano, and 3.62 µg/cm² and 19 µg/cm² in DEET, respectively (Table 4). In the case of comparing ED₅₀ and ED₉₀ in essential oils and DEET, the results showed that, ED₅₀ and ED₉₀ in *E. globulus* Nano and *M. piperita* Nano were significantly lower than ED₅₀ and ED₉₀ for essential oils *E. globulus* and *M. piperita*. Comparison of 95% CI also showed that ED₅₀ and ED₉₀ in DEET were much lower than ED₅₀ and ED₉₀ for *M. piperita*, *M. piperita* Nano, *E. globulus*, and *E. globulus* Nano (Table 5 and Figure 3). The equations and dose-response lines for tested compounds are shown in Figure 3. Logarithmic scale was used for x (concentration) and probit scale was used for y (percent of repelled insects). Slope of the related lines indicate the tested population of *An. stephensi* was homogenous.

Table 4. Determination of the repelling indicators (ED₅₀ and ED₉₀) of DEET, normal and nanoemulsion essential oils against *Anopheles stephensi* on human volunteers.

Repellents	Number of mosquitos	ED ₅₀ (µg/cm ²)	95% CI (µg/cm ²)	ED ₉₀ (µg/cm ²)	95% CI (µg/cm ²)
<i>Eucalyptus</i>	300	36.10	(28.70-48.01)	199	(126-445)
Nano- <i>Eucalyptus</i>	300	18.50	(14.65-23.23)	98	(64-203)
<i>Mentha</i>	300	29.10	(23.36-36.06)	139	(94-266)
Nano- <i>Mentha</i>	300	19.39	(15.35-23.99)	94	(65-180)
DEET	300	3.62	(2.68-4.55)	19	(13-38)

Table 5. Equation of regression line and χ^2 (df)± SE for effectiveness of DEET, and normal and nanoemulsion essential oils against *Anopheles stephensi* on human volunteers.

Repellents	Equation of regression line	χ^2 (df)± SE	<i>P</i> -value
<i>Eucalyptus</i>	y=2x+3	0.25(2)±0.26	0.88
Nano- <i>Eucalyptus</i>	y=2x+2.6	0.24(2)±0.26	0.88
<i>Mentha</i>	y=2.5x+3.5	1.22(2)±0.27	0.54
Nano- <i>Mentha</i>	y=1.6x+2.3	0.78(2)±0.27	0.67
DEET	y=1.6x+0.83	0.31(2)±0.27	0.88

4. Discussion

Of 33 *Anopheles* species known from Iran, seven species play an important role in malaria transmission in Iran, of which *An. stephensi* is one of the most important species[24-26]. The main method to control mosquitoes is the use of synthetic insecticides that have harmful effects on human and animal health and the environment. Therefore, the use of natural products, including essential oils, is of paramount importance as being environmentally compatible and degradable[27,28]. The results of this study showed that nanoformulated *M. piperita* 50% essential oil increased its protection time from 2.89 h to 4.17 h and the failure time from 3.99 h to 5.09 h. Also, the protection time of *E. globulus* 50% essential oil rose from 0.96 h to 5.51 h and its failure time from 2.06 h to 6.51 h. Effective dose determination revealed that the nanoformulated essential oils also reduced the ED₅₀ values of these compounds from 29.10 µg/cm² to 19.39 µg/cm², and ED₉₀ values from 139 µg/cm² to 94 µg/cm² in *M. piperita* essential oil. In *E. globulus* essential oil, ED₅₀ decreased from 36.10 µg/cm² to 18.50 µg/cm², and ED₉₀ from 199 µg/cm² to 98 µg/cm². In a similar study[29], a nanoemulsion was prepared from the essential oil of citronella plant and the repellent effect of this nanoemulsion compound was compared with the normal compound. The results showed that addition of glycerol improved the physical appearance and stability of the nanoemulsion, and also increased the protection time of nanoemulsion compounds, which is completely in line with those of this research. In another study by Nuchuchua *et al*[8], the results showed that preparation of nanoemulsion essential oils from citronella, hairy basil, and vetiver could enhance the protection time of these essential oils up to 4.7 h. Nuchuchua *et al*. [8] reported that the droplet size of nanoemulsion and the composition of repellents play an important role in determining the protection time, and that a smaller droplet size of nanoemulsion improves physical stability, and improves protection time and the efficacy of the compounds due to forming an integrated coating on the human skin.

In this study, protection time of 6.10 h and failure time of 7.12 h were obtained for DEET 25%. Tavasoli *et al.* 2011[23] reported a protection time of 6.23 h and a failure time of 7.30 h, which are almost the same as those in here. Fradin and Day[30] reported that protection time, failure time, and DEET depend on the concentration, formulation, and the tested mosquito species and can vary in different conditions[31].

ED₅₀ and ED₉₀ values of 3.62 µg/cm² and 19 µg/cm², respectively, were obtained for DEET in this study. Tavasoli *et al.* 2011[23] reported ED₅₀ and ED₉₀ values of 2 µg/cm² and 9 µg/cm², respectively, for DEET. Vatandoost and Hanafi-Bojd[6] found an ED₅₀ value of 5 µg/cm² for DEET, which is approximately the same as that of this study. Therefore, according to the results obtained

from this study, it can be concluded that *M. piperita* Nano and *E. globulus* Nano can be a good alternative to DEET and other chemical compounds.

Overall the results of this study show that preparation of nanoemulsions from *M. piperita* essential oil increased its protection time about two times and reached its to 4.2 h; also about the protection time of *E. globulus* essential oil, this increase was fivefold and reached it to 5.51 h. Effective dose determination revealed that the preparation of nanoemulsions from essential oils reduced the ED₅₀ values of them, hence, *M. piperita* Nano and *E. globulus* Nano can be a good alternative to DEET and other chemical compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

Authors' contributions

RM. prepared the manuscript; RM. MK. did the literature search; RM. MK. MN. made clinical studies; RM. MK. MN. SK. did concepts, design, definition of intellectual content, data acquisition, statistical analysis, manuscript editing, manuscript review, guarantor.

References

- [1] Panneerselvam C, Murugan K, Kovendan K, Mahesh Kumar P, Subramaniam J. Mosquito larvicidal and pupicidal activity of *Euphorbia hirta* Linn. (Family: Euphorbiaceae) and *Bacillus sphaericus* against *Anopheles stephensi* Liston. (Diptera: Culicidae). *Asian Pac J Trop Med* 2013; **6**: 102-109.
- [2] World Health Organization. Global malaria program. *World malaria report*. Geneva: WHO; 2015.
- [3] Shinzawa N, Ishino T, Tachibana M, Tsuboi T, Torii M. Phenotypic dissection of a *Plasmodium*-refractory strain of malaria vector *Anopheles stephensi*: The reduced susceptibility to *P. berghei* and *P. yoelii*. *PLoS One* 2013; **8**(5): e63753.
- [4] WHO. *Global vector control response 2017–2030*. Geneva: WHO; 2017.
- [5] Soltani A, Vatandoost H, Oshaghi MA, Ravasan NM, Enayati AA, Asgarian F. Resistance mechanisms of *Anopheles Stephensi* (Diptera: Culicidae) to temephos. *J Arthropod Borne Dis* 2015; **9**(1): 71-83.
- [6] Vatandoost H, Hanafi-Bojd A. Current resistant status of *Anopheles stephensi* Liston to different larvicides in hormozgan province, southeastern Iran, 2004. *Pak J Biol Sci* 2005; **8**: 1568-1570.
- [7] Logan JG, Stanczyk NM, Hassanali A, Kemei J, Santana AEG, Ribeiro KAL, et al. Arm in cage testing of natural human-derived mosquito

- repellents. *Malar J* 2010; **9**: 239.
- [8] Nuchuchua O, Sakulku U, Uawongyart N, Puttipipatkachorn S, Soottitantawat A, Ruktanonchai U. *In vitro* characterization and mosquito (*Aedes aegypti*) repellent activity of essential-oils-loaded nanoemulsions. *AAPS PharmSciTech* 2009; **10**(4): 1234-1242.
- [9] Maia FM, Moore SJ. Plant-based insect repellents: A review of their efficacy, development and testing. *Malar J* 2010; **10**(Suppl 1): S11.
- [10] Kim J, Kang C, Lee J, Kim Y, Han H, Yun HK. Evaluation of repellency effect of two natural aroma mosquito repellent compounds, citronella and citronellal. *Entomological Research* 2005; **35**: 117-120.
- [11] Trongtokit S, Rongsriyam Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res* 2005; **19**: 303-309.
- [12] Jaenson TG, Garboui S, Palsson K. Repellency of oils of lemon eucalyptus, geranium, and lavender and the mosquito repellent Mygga natural to *Ixodes ricinus* (Acari: Ixodidae) in the laboratory and field. *J Med Entomol* 2006; **43**: 731-736.
- [13] Tawatsin A, Wratten SD, Scott RR, Thavara U, Techadamrongsin Y. Repellency of volatile oils from plants against three mosquito vectors. *J Vector Ecol* 2001; **26**: 76-82.
- [14] Khoobdel M, Ahsaei SM, Farzaneh M. Insecticidal activity of polycaprolactone nanocapsules loaded with *Rosmarinus officinalis* essential oil in *Tribolium castaneum* (Herbst). *Entomological research* 2017; **47**(3): 175-184.
- [15] Osanloo M, Amani A, Sereshti H, Abai MR, Esmaili F, Sedaghat MM. Preparation and optimization nanoemulsion of Tarragon (*Artemisia dracunculus*) essential oil as effective herbal larvicide against *Anopheles stephensi*. *Ind Crop Prod* 2017; **109**: 214-219.
- [16] Buranasuksombat U, Kwon YJ, Turner M, Bhandari B. Influence of emulsion droplet size on antimicrobial properties. *Food Sci Biotechnol* 2011; **20**(3): 793-800.
- [17] McClements DJ. Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter* 2012; **8**: 1719-1729.
- [18] Ostertag F, Weiss J, McClements DJ. Low-energy formation of edible nanoemulsions: Factors influencing droplet size produced by emulsion phase inversion. *J Colloid Interface Sci* 2012; **388**: 95-102.
- [19] Solans C, Solé I. Nano-emulsions: Formation by low-energy methods. *Curr Opin Colloid & Interf Sci* 2012; **17**: 246-254.
- [20] Duarte JL, Amado JRR, Oliveira AEMFM, Cruz RAS, Ferreira AM, Souto RNP, et al. Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. *Braz J Pharmacog* 2015; **25**: 189-192.
- [21] Oliveira AE, Duarte JL, Cruz RA, Souto RN, Ferreira RM, Peniche T, et al. *Pterodon emarginatus* oleoresin-based nanoemulsion as a promising tool for *Culex quinquefasciatus* (Diptera: Culicidae) control. *J Nanobiotechnology* 2017; **15**(1): 2.
- [22] Pirmohammadi M, Shayeghi M, Vatandoost H, Abaei MR, Mohammadi A, Bagheri A, et al. Chemical composition and repellent activity of *Achillea vermiculata* and *Satureja hortensis* against *Anopheles stephensi*. *J Arthropod Borne Dis* 2016; **10**(2): 201-210.
- [23] Tavassoli M, Shayeghi M, Abai M, Vatandoost H, Khoobdel M, Salari M, et al. Repellency effects of essential oils of Myrtle (*Myrtus communis*), Marigold (*Calendula officinalis*) compared with DEET against *Anopheles stephensi* on human volunteers. *Iran J Arthropod Borne Dis* 2011; **5**(2): 10-22.
- [24] Service MW. *A guide to medical entomology*. London: MacMillan; 1980, p. 226.
- [25] Vatandoost H, Sanei dehkordi A, Sadeghi S, Davari B, Karimian F, Abai M, et al. Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima* Mozaffarian essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae). *Exp Parasitol* 2012; **132**(4): 470-474.
- [26] Sedaghat MM, Harbach RE. An annotated checklist of the *Anopheles* mosquitoes (Diptera: Culicidae) in Iran. *J Vector Ecol* 2005; **30**(2): 272-276.
- [27] Vatandoost H, Khazani A, Rafinejad J, Khoobdel M, Kebriai-Zadeh A, Abai M. Comparative efficacy of neem and dimethyl phthalate (DMP) against malaria vector, *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Med* 2008; **1**(3): 1-6.
- [28] Murugan K, Chandrasekar R, Panneerselvam C, Madhiyazhagan P, Subramaniam J, Dinesh D, et al. *Nano-insecticides for the control of human and crop pests. Short views on insect genomics and proteomics: Insect proteomics*. Switzerland: Springer International Publishing; 2016, p. 229-251.
- [29] Sakulku U, Nuchuchua O, Uawongyart N, Puttipipatkachorn S, Soottitantawat A, Ruktanonchai U. Characterization and mosquito repellent activity of citronella oil nanoemulsion. *Int J Pharm* 2009; **372**(1-2): 105-111.
- [30] Fradin MS, Day GF. Comparative efficacy of insect repellents against mosquito bites. *New England J Med* 2002; **47**: 13-18.
- [31] Klun JA, Khirimiyan A, Debboun M. Repellent and deterrent effects of SS220, Picaridin, and DEET suppress human blood feeding by *Aedes aegypti*, *Anopheles stephensi*, and *Phlebotomus papatasi*. *J Med Entomol* 2006; **43**: 34-39.