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GC-MS analysis and anti-mosquito activities of *Juniperus virginiana* essential oil against *Anopheles stephensi* (Diptera: Culicidae)

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ABSTRACT

Objective: To investigate phytochemicals present in the essential oil from aerial parts of eastern red cedar, *Juniperus virginiana* (*J. virginiana*) L. (Cupressaceae) and to determine its killing and repellent activities against larvae, pupae, and adults of the Asian malaria mosquito, *Anopheles stephensi* (Diptera: Culicidae).

Methods: *J. virginiana* essential oil was extracted by hydrodistillation, and its chemical composition was determined by gas chromatography-mass spectrometry. Seven different logarithmic concentrations of *J. virginiana* essential oils were used in larvicidal and pupicidal assays. *J. virginiana* essential oils-impregnated bed nets were applied in a designed animal module to test excito-repellent activity against adult mosquitoes.

Results: Fourteen constituents corresponding to 99.98% of *J. virginiana* essential oils were identified. Five main components were terpinen-4-ol (25.21%), camphor (19.89%), E-3-hexen-1-ol (13.30%), γ-terpinene (7.86%), and l-menthone (2.27%). The LC_{50} and LC_{90} values against larvae of the *Anopheles stephensi* were 11.693 and 66.140 ppm and for pupae were 9.640 and 40.976 ppm, respectively. In excito-repellency assay, *J. virginiana* essential oils-impregnated bed nets provided an average of 54.63% protection for guinea pig and 45.37% mortality for the mosquitoes.

Conclusions: Four monoterpenes and one leaf alcohol were identified by gas chromatography-mass spectrometry. *J. virginiana* essential oils showed potent larvicidal, pupicidal, adulticidal, and repellent activities against *Anopheles stephensi* at acceptable concentrations. Evaluation of bioactivity of identified chemicals (alone or in combination) will provide new eco-friendly substances for mosquito-management programs.

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1. Introduction

Mosquitoes (Diptera: Culicidae) are considered among the most dangerous organisms in the world. They are vectors of notorious human diseases, including malaria, zika, chikungunya, and dengue infecting around 700 million people worldwide and causing death of roughly one million annually[1]. Currently, more than 50% of the world's populations share their in/outdoor places by at least one mosquito species[2]. Globally, 41 species of *Anopheles* species are documented to be the main vectors of malaria infection[3], with two of the major tropical species being *Anopheles gambiae* (prevalent in Africa) and *Anopheles stephensi* (*An. stephensi*) (prevalent in Asia)[4]. *An. stephensi* is the major vector of both *Plasmodium falciparum* and *Plasmodium vivax* parasites ranging from Middle East to the Indian subcontinent and China[5]. It is also a principal vector of urban and rural malaria in the south and southeast of Iran[6–10]. Vector control is a main way to prevent and reduce malaria transmission[11,12]. However, high reproduction rates (70-200 eggs per gonotrophic cycle), relatively small sizes, and living in both aquatic and terrestrial environments[13] make mosquito control more difficult compared to other vectors. To date, an effective vaccine against malaria is not available[14] and the prevention is solely focused on three principles of reducing the larval source, decreasing populations of adult mosquitoes, and personal protection[11]. Each preventive method has its own advantages and disadvantages. Larval source management comprising habitat modification/manipulation, larviciding, and biological control is a major financial and technical responsibility, necessitating both community mobilization and long-term political commitment[15]. Either indoor residual spraying or area spraying (fogging) will be helpful in emergency conditions such as epidemics[16]. Nevertheless, indoor residual spraying may bring the pesticides more close to humans[17] and the area spraying should have repeated and properly scheduled corresponding to the peak time of adult mosquito activity[18]. People at risk of malaria can be protected by using window screens, long-lasting insecticidal nets, repellents, and wearing proper clothes[19]. Repellents will be effective in reducing malaria transmission in travelers and in areas where mosquitoes are active outdoors in the evening while long-lasting insecticidal nets are recommended for people in the endemic area[20]. Chemical pesticides used in the malaria vector control are harmful to the environment and human health and induce resistance in a number of mosquito species[12,21–23]. Therefore, eco-friendly methods have been recently considered to enhance vectors control efficiency[23–26] and decrease their side effects. Plant essential oils (EOs), the first generation of herbal pesticides, are known as green pesticides[22,27,28]. They show anti-insect activities including insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory, and anti-vector properties[27]. However, their main function is insecticidal activity[29,30]. Normally, EOs can be inhaled, ingested, or skin absorbed by insects. They are lipophilic and primarily penetrate through the chitinous exoskeleton to intervene with biochemical, basic metabolic, physiological, and behavioral functions of the target insects[31]. They act rapidly against some pests and degrade rapidly in the environment[32]. The genus *Juniperus* L. (Cupressaceae) with about 70 known species is distributed worldwide as native or non-indigenous plants[33]. In Mediterranean Basin, different parts of the *Juniperus* spp. have been used in embalming, medicine, and cosmetics for a long time[33]. In recent years, antimicrobial and insecticidal properties of the *Juniperus* species have been the focus of attention[34–38]. The aim of this study was to analyze the chemical compositions and to evaluate both larvicidal/pupicidal and adult repellent potency of a *Juniperus* sp. EO against *An. stephensi*.

2. Materials and methods

2.1. Collection and preparation of EO from plant materials

The aerial green parts of a *Juniperus* sp. were collected in May 2017 from the green spaces in the Production and Research Complex of the Institute Pasteur of Iran (IPI;51° 3′ 44″ N, 35° 45′ 49″ E and 1 330 m above sea level). The plant species were identified and authenticated as Eastern Redcedar, *Juniperus virginiana* (*J. virginiana*) Linnaeus 1753 (Cupressaceae), by Prof. Valiollah Mozaffarian from Research Institute of Forests and Rangelands, Tehran, Iran.

To obtain EO, the plant specimens were washed twice thoroughly with distilled water, immediately after the collection in the morning. About 100 g of the plant materials were ground, mixed with 500 mL of distilled water and then hydro-distilled using a Clevenger apparatus with continuous extraction for 2 h. The yielded EO (~ 0.5 - 0.7 mL) was separated from water, dried over anhydrous sodium sulfate and stored at 4 ℃.

2.2. Gas chromatography–mass spectrometry (GC–MS)

The GC-MS analysis was performed on a GC (7890B, Agillent, USA) equipped with a mass spectrometer (5977B, Agillent, USA). A capillary column (VF-1 ms, 15 m, 0.25 mm, 0.25 μm) was used for the analysis. Helium served as the carrier gas, and all the samples were analyzed under the following conditions: initial temperature at 40 ℃ for 4 min, ramp up at 15 ℃/min to 150 ℃ and remained for 2 min, then increased to 270 ℃ with a ramp up of 15 ℃/min to, injector = 180 ℃, volume = 5.0 μL, split = 20, source temp = 250 ℃, full scan. Compounds were identified by comparison of their respective mass spectra, retention indices (Kovats index), and relative abundance of acceptance match criteria with those of standards and by comparing with the NIST and Wiley mass spectral data system/library.

2.3. Mosquito sources

The source of *An. stephensi* mosquitoes used in this study was from Chabahar City, Sistan and Baluchestan Province, which is the endemic focus of malaria in Iran. This strain was rearing alongside with others in the insectarium of School of Health located at Shiraz University of Medical Sciences. The colonies were held routinely at (27 ± 2) ℃ and (65 ± 5)% relative humidity with 12:12 light/dark photoperiodicity. The immature stages including four larval instars and pupae were reared in the plastic trays (35 cm × 20 cm × 5 cm), one fifth-filled with chlorine-free tap water. Larvae were fed on Tetra Goldfish food (Tetra GmbH, Germany) until pupation. Emerged adults were collected from the trays using electric aspirator and

released into adult rearing cages (50 cm × 50 cm × 50 cm). Adult mosquitoes were fed on a 10% sucrose solution *ad libitum* and blood fed on white lab mouse twice a week. Deposited eggs were collected in small white bowls, and after hatching, larvae were transferred into separated trays to produce a new generation.

2.4. Larvicidal bioassay

According to the standard methods described in the literatures[39,40], 4th instar larvae of the *An. stephensi* were exposed to seven (2.5 to 160 ppm) serially diluted concentrations of the EO, for 24 h. Initially, the *J. virginiana* essential oil (JVEO) was dissolved in ethanol 99% as stock, and subsequent solutions were prepared by stock dilution. For each treatment, 99 mL of the dechlorinated water containing 0.000 7% Tween-80 was added to 250 mL glass beaker. Next, 1 mL of each concentration of the JVEO was added to a beaker to make up 100 mL of test solution. The oil-ethanol-water solution was stirred gently for 30 s using a glass rod. Two beakers, one containing tap water mixed with Tween-80 and 1% ethanol and the other composed of untreated dechlorinated water were set as controls. A minimum of 25 healthy larvae were collected by a strainer with fine mesh and then were gently transferred into the beakers. The bioassay was done in a test room with (24 ± 1) ℃ and (50 ± 5)% relative humidity. Observation on larval mortality was recorded after 24 hours of exposure, and percentage of mortality was reported for the average of four replicates. Larvae were considered as dead when they did not respond to touching with a fine rode.

2.5. Pupicidal bioassay

The pupicidal potency of the JVEO was evaluated as described previously[39,40]. The test conditions and concentrations were the same as those stated in the larvicidal bioassay. The difference was that ten two-day pupae of *An. stephensi* were introduced into an aqueous medium containing 99 mL of dechlorinated water, emulsifier Tween-80, and 1 mL of an appropriate dilution of essential oil. After 24 h, the number of dead pupae, pupae with incomplete emerge, live pupae, and live adults were enumerated. The pupal mortality was calculated based on the number of dead pupae and incomplete emerges (which was considered presumably dead) in four replicates.

2.6. Excito-repellency bioassay

Mosquito excito-repellency test was conducted in a fabricated device consisting of a restrainer and two test chambers. A triparite animal restrainer was prepared using Plexiglas materials with dimensions of 37 cm × 10 cm: two dark and bright chambers at both ends, respectively for restriction of animal head and legs and the middle part for repellency assay with 25-mesh wire screen (9 cm × 6 cm) (Figure 1, A, D, and E)[41]. The test chambers, made from Plexiglas, were the main parts of the devise in which the large (38 cm × 38 cm × 38 cm) and small (20 cm × 20 cm × 20 cm) boxes were applied to test the repellency and irritability effects of the given chemicals/EOs (Figure 1, B, C, F, and G)[41]. Mosquitoes could be released into the exposure chamber by a small entry opening (diameter = 10 cm) equipped with a netting sleeve (25 cm length). The fabricated module facilitated the study of the behavioral biology of mosquito vectors with a minimum visual error. The device is designed in a way to take ethical considerations into animal experiments

Prior to the test, polyester bed nets (20 cm × 20 cm) were impregnated with crude JVEO using the dipping method described by Rozendaal[42]. After drying, nets were used for repellency test. The control nets were left untreated. During the test time, a medium size guinea pig was restrained. Next, the restrainer was covered by JVEO impregnated bed net and then guinea pig was exposed to the bites of mosquitoes. The individual 5- to 7-day-old female *An. stephensi* were starved for 24 h and then were released into the exposure chamber where the restrainer located. Observations on the number of blood-fed mosquitoes were recorded at 45 min post exposure. Live and dead (or knocked-down) mosquitoes were enumerated as well. The exit trap was checked for the possible entry of mosquitoes. Each test was performed in triplicates and the percentage of protection was calculated by the following equation: % protection= (Nm/Nt) × 100; where Nm is the mean number of unfed females in the treatment group, and Nt is the total number of mosquito.

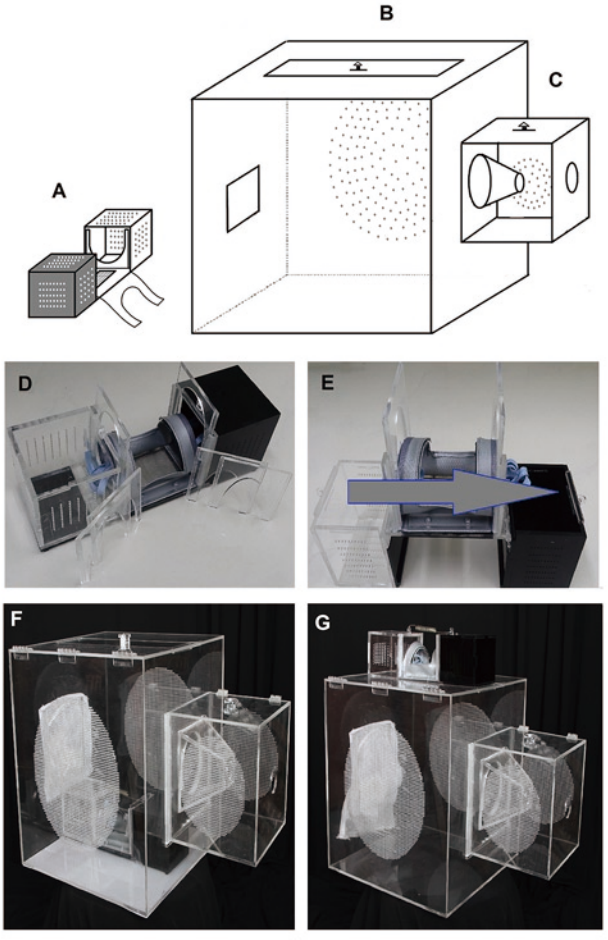

Figure 1. The device used for repellency assay. A: schematic illustration of the tripartite restrainer, B and C: schematic illustration of the large and small test chambers, D: three different sized holders to adjust animal body size with the restrainer, E: the direction that a guinea pig can be confined in the restrainer, F: the restrainer can be applied inside the large chamber, and G: on top of it.

Table 1. Chemical composition of *J. virginiana* essential oils characterized by GC/MS.

Peak No.	Component	MW•	Retention time	Kovats index	% Chemical compositions
1	(E)-3-Hexen-1-ol	100	5.159	589	13.30
2	ND*	208	5.371	593	5.46
3	ND*	296	7.757	832	3.91
4	1,8-Cineol	154	7.963	851	7.37
5	γ -Terpinene	136	8.577	895	7.86
6	α -Ocimene	136	9.090	965	5.55
7	Camphor	152	9.690	997	19.89
8	l-Menthone	154	10.120	1 102	2.27
9	Terpinen-4-ol	154	10.314	1 116	25.21
10	(+)-4-Carene	136	10.509	1 131	1.44
11	ND*	154	11.001	1 169	2.05
12	(−)-Bornyl acetate	196	11.985	1 337	1.77
13	ND*	915	22.914	1 863	1.63
14	ND*	915	23.830	1 933	2.27

•MW: molecular weight, *ND: not determined.

Table 2. Probit regression line parameters of *J. virginiana* essential oils against larvae and pupae of *An. stephensi*.

Activity	A	B±SE	LC_{50} (95% *CI*)	LC_{90} (95% *CI*)	χ^2 (*df*)	*P* value
Larvicidal	-5.3	1.703±0.113	11.693 (8.907-15.048)	66.140 (46.187-110.356)	8.123 7(5)	<0.05
Pupicidal	-3.61	2.039±0.209	9.640 (7.704-11.902)	40.976 (30.635-61.086)	1.815 7(5)	<0.05

Abbreviations: A, intercept; B, slope; SE, standard error; LC_{50}, 95 % *CI*, lethal concentration causing 50% mortality and its 95% confidence interval; LC_{90}, 95% *CI*, lethal concentration causing 90% mortality and its 95% confidence interval; χ^2, heterogeneity about the regression line; df, degrees of freedom.

2.7. Statistical analysis

Mortality data after 24 hours of exposure to different concentrations of JVEO were subjected to Probit analysis to determine lethal concentrations (LC_{50} and LC_{90}) of larvae and pupae. The data were corrected by Abbott's formula if mortality in control beakers was 2%-5%. The *P* values less than 0.05 represented correlations between JVEO doses and mortalities.

2.8. Ethical statement

Study procedure was done based on national regulations and ethical considerations in animal experiments. The research committee and institutional Ethics Committee of the Pasteur Institute of Iran approved this project (No. 1563).

3. Results

3.1. Yields and chemical composition of JVEO

The hydrodistillation of the JVEO aerial green parts provided oil in 0.14% (w/w) yield on fresh weight material. The GC-MS analysis revealed the presence of 14 constituents in the JVEO corresponding to 99.98% of the total oil (Figure 2 and Table 1). Five chemical compositions of terpinen-4-ol (25.21%), camphor (19.89%), E-3-hexen-1-ol (13.30%), γ -terpinene (7.86%), and l-menthone (2.27%) were identified with a quality of ≥ 95%. Four components of 1,8-cineol (7.37%), α -ocimene (5.55%), (−)-bornyl acetate (1.77%), and (+)-4-carene (1.44%) were identified with a quality of ≥ 83%. It should be noted that the name of other five constituents with qualities lower than 83% was not determined (Table 1).

3.2. Larvicidal and pupicidal activity of JVEO

Probit regression line parameters of JVEO against larvae and pupae of *An. stephensi* are shown in Table 2. In both larvicidal and pupicidal assays, the mortality rates in the control groups were lower than 5% in all concentrations, so the corrections were not applied. The LC_{50} and LC_{90} values against larvae of *An. stephensi* were 11.693 and 66.140 ppm and for pupae were 9.640 and 40.976 ppm, respectively. The JVEO showed to be more effective against pupae than larvae, as illustrated in Figure 3.

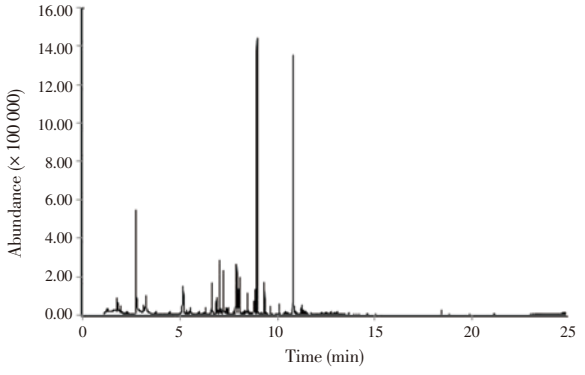

Figure 2. GC/MS chromatogram of *J. virginiana* essential oils.

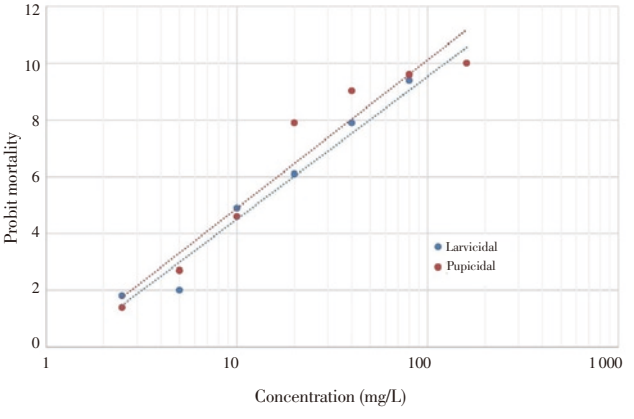

Figure 3. Probit regression line of larvae and pupae of *An. stephensi* exposed to different concentrations of *J. virginiana* essential oils.

Table 3. Repellency test result of *J. virginiana* essential oils against *An. stephensi* under laboratory conditions.

Groups	Blood fed [*n*(%)]	Unfed [*n*(%)]	Mortality [*n*(%)]	%Protection
Treatment 1	0 (0)	7 (58.3)	5 (41.7)	58.3
Treatment 2	0 (0)	7 (50.0)	7 (50.0)	50.0
Treatment 3	0 (0)	10 (55.6)	8 (44.4)	55.6
Control	12 (85.7)	0 (0)	2 (14.3)	0

3.3. Excito-repellency activity

The crude JVEO provided an average of 54.63% protection to the guinea pig against *An. stephensi* bite. There was not any blood-fed mosquito in three treatments. Instead, many dead mosquitoes were found inside the test chambers indicating killing effects of JVEO rather than repellency. The JVEO caused an average of 45.37% mortality in the tested adults. There was not any mosquito in the small exit box. Detailed information is presented in Table 3.

4. Discussion

Despite remarkable scientific progress, mosquito-borne diseases have still remained as a major threat to humankind[43]. Therefore, there is an urgent need for novel bioactive natural products, especially from plant resources (which are fed by both larval and adult stages of mosquitoes) to overcome problems caused by chemical pesticides. Plant species containing attractant and/or repellent compounds to insects can be utilized in every anti-mosquito program; however, the repulsive group is more applicable. Aromatic plants such as *Juniperus* spp., which is naturally avoided by insect species, will be appropriate candidates. In this regard, we investigated the chemical composition of JVEO by GC-MS and tested its killing and repellent activities against both aquatic and terrestrial stages of *An. stephensi*.

Chemical components of the JVEO were dominated by five components, including four monoterpenes and one leaf alcohol (Table 1). Terpinen-4-ol, the most abundant component, is a monoterpene with a wide range of anti-microbial, anti-parasitic, antioxidant, anti-inflammatory, anti-cancer and insecticidal activities. It was found to be the primary active ingredient of Melaleuca (tea tree), Meristic (nutmeg), and Juniper (coniferous) species[44-47]. In various studies, fumigant, antifeedant and insecticidal activities of terpinen-4-ol have been approved in target insects[48-51]. Terpinen-4-ol is the main component of EOs like *Melaleuca alternifolia* that could significantly arrest the activity of glutathione S-transferase, carboxylesterase and acetylcholinesterase enzymes[51].

Camphor, another frequent monoterpene in JVEO, is used for its scent, as a cooking ingredient, an embalming fluid, for medicinal purposes, and in religious ceremonies[52]. It has been applied in the treatment of rheumatism, bronchitis, muscle pain, asthma, sprains, and as a cold remedy[53]. Also, camphor was effective alone or in combination with others against some serious diseases. 714-X is a camphor-based drug, which is effective for breast and prostate cancer[54]. Padma 28 is another drug based on camphor formula and effective against chronic inflammatory diseases[55]. Fumigant, repellence and insecticidal activities of camphor were measured on pest insects as well[56-58]. Additionally, it has been proposed that the synergistic toxicity of dual mixture of camphor and 1,8-cineole is correlated with the degree of penetration through the insect's exoskeleton[59].

E-3-hexen-1-ol (or leaf alcohol) is a colorless oily liquid with an intense grassy-green odor, particularly emitted from several plant species and many fruits[60]. Actually, it is applied by both herbivores and natural enemies to trace the host and host prey in multitrophic interactions[61]. Hence, this compound can be applied in integrated pest management strategies.

The γ-terpinene is another monoterpene isolated from a variety of plant sources. This terpene has been shown to possess antioxidant[62,63], antimicrobial[62], insecticidal[64] and antifeedant[50] activities. The acaricidal and knockdown activity of γ-terpinene was revealed against adult ticks of *Hyalomma marginatum* (Acari: Ixodidae)[65]. Similar to organochlorines and pyrethroids, this terpene may act on voltage-gated sodium channels, which are essential for the beginning and spread of the action potential in the nervous system[66].

Menthone is generally used in flavor compositions. It is found with menthol in many EOs, including peppermint, geranium, and other plants. Menthone has shown antimicrobial and antioxidant activities[44,67,68]. The insecticidal potency of menthone has been approved in control of rice weevil, *Sitophilus oryzae*[69]. The mosquitocidal activity of menthol derivatives has been shown to be due to the presence of the major aroma compounds such as menthol and not due to minor components, *e.g.* menthone[70].

Recently, Stewart and colleagues[71] determined the chemical composition of the EOs from different parts of *J. virginiana* using GC-MS analysis[71]. Contrary to our study results, they found higher concentrations of limonene in the berries, α-pinene in the bark, and safrole, and methyl eugenol in the leaves[71]. The reason for this discrepancy may be due to ecological conditions, the mix preparation of berries and leaves, as well as the difference in the season of the plant collection.

The results of current study could validate larvicidal and pupicidal activities of different concentrations of JVEO against *An. stephensi*. The LC_{50} values against larvae and pupae were 11.693 and 9.640 ppm, respectively. According to the guideline developed for the larvicidal activity of plant EOs[40], JVEO is ranked in the third category requiring more attention and research. In this study, the mortality of mosquito larvae and pupae was completely dosage-dependent. However, the effect of JVEO on pupae was 0.01 time more toxic than on larvae. This discrepancy may be related to the specimens difference in terms of sex, age, size, and physiological status as indicated in other studies[72].

In several studies, EOs extracted from *Juniperus* species have been evaluated against mosquito species. Prajapati and colleauges[29] investigated bioactivities of 10 EOs, including *Juniperus macropoda* against *An. stephensi*, *Aedes aegypti* (*Ae. aegypti*), and *Culex quinquefasciatus* (*C. quinquefasciatus*) and showed that they are highly effective larvicides (LD_{95}: 110.2-204.8 μg/mL)[29]. Amer and Mehlhorn[35] investigated the larvicidal activity of the oils from 41 plant species, including *J. virginiana* and *Juniperus communis* (*J. communis*) against 3rd instar larvae of three above-mentioned species. The LC_{50} values of the three mosquito species when exposed to *J. virginiana* oil have been ~ 10 ppm for both *An. stephensi* and

C. quinquefasciatus and < 5 ppm for *Ae. aegypti*. In comparison, the LC_{50} values for *J. communis* were respectively reported as ~ 100, 50, and 10 ppm for *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus*, respectively. Thus, *An. stephensi* was reported to be more resistant than two other mosquito species to *J. communis*[35]. Vourlioti-Arapi and colleagues[37] studied EOs from various parts of the six indigenous *Juniperus* spp. against 3rd and early 4th instar larvae of *Culex pipiens*. They found that the EO of *Juniperus drupacea* obtained from the wood part is the best larvicide with an LC_{50} value of 26.47 mg/L[37]. Larvicidal activity of *Juniperus procera* EO was also studied against *Anopheles arabiensis* under laboratory and semi-field conditions. The LC_{50} values for both conditions were reported to be 14.42 and 24.51 mg/L, respectively[73].

Uniyal *et al*[38] examined an EO from *J. communis* against 3rd instar larvae of *Ae. aegypti* and reported a 36% mortality after 24 hours of exposure in 500 mg/L (LC_{50} = 276.076)[38]. Regardless of the differences between *Juniper* and target mosquito species, the larvicidal results of the current study were in line with previous studies and even provided some better results. However, no study was found to compare the pupicidal results.

The last part of the study was preliminary analysis on the bioactivity of JVEO against adults *An. stephensi*. The repellency activity of JVEO was tested using a module made from transparent Plexiglas. The device enabled the operator to easily follow landing, biting, or avoiding behaviors of the tested mosquitoes. The device, which was patterned from the houses in malaria-endemic areas, can be used for concomitant assessment of irritability and repellency, the efficiency of synthetic or natural repellents applied on the body/clothes, or as a blood feeding apparatus.

We used the crude JVEO in repellency assay (without irritancy) and found an average of 54.63% protection up to 45 min for guinea pig against *An. stephensi* bite. Amer and Mehlhorn[74] investigated the repellency effect of oils from 41 plant species, including two *Juniperus* species (*J. virginiana* and *J. communis*) against *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus* using the skin of human volunteers. They reported weak (37.8% and 43.2%), mild (38.1% and 76.2%), and great (100% and 100%) repellency effect against *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus*, respectively[74].

The results of our study together with Amer and Mehlhorn's study indicate that *J. virginiana* has repellency property against adult mosquitoes; however, these results are not comparable due to the difference in mosquito's and host species tested. The JVEO was showed adulticidal (~ 45.37% mortality) against *An. stephensi* rather than repellency effect. EOs having this property are important since they can protect human hosts from mosquito bites, and may cause pathogen depletion from the mosquito populations.

Herein, we identified four monoterpenes (terpinen-4-ol, camphor, γ -terpinen, and l-menthone) and one leaf alcohol (E-3-hexen-1-ol) from JVEO, which presumably were involved in the biological activities of JVEO. The oil showed potent larvicidal, pupicidal, adulticidal, and repellent activities against *An. stephensi*. This oil may be a candidate for the development of new, safe, effective, and inexpensive control measures such as nanoemulsions or nanocapsules for different life stages of *An. stephensi*. Evaluation of bioactivity of identified chemicals (alone or in combination) will provide lead products for mosquito control programs in field trials.

Conflict of interest statement

Authors declare that there are no competing interests.

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