

## Original Article

# Evaluation of Repellency Effect of Essential Oils of *Satureja khuzestanica* (Carvacrol), *Myrtus communis* (Myrtle), *Lavendula officinalis* and *Salvia sclarea* using Standard WHO Repellency Tests

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### Abstract

**Background:** Using special lotions and repellent sprays on skin is one of the effective methods to prevent Arthropods biting which was verified in this study.

**Methods:** Essential oils of four plants (*Satureja khuzestanica*, *Salvia sclarea*, *Lavendula officinalis* and *Myrtus communis*) were separately extracted by Clevenger used hydro distillation method. Then separated solutions with 10%, 20% and 40% concentrations of essential oils of plants in 99.6 % ethanol were prepared. WHO guidelines for efficacy testing of mosquito repellents for human skin were used on different concentrations of essential oils of plants, traditional repellents (DEET, 50% and 33%) as positive control, and ethanol 99.6% and naked hands as negative controls.

**Results:** In negative control groups, the number of bits were comparable ( $P= 0.42$ ) and had decreasing time trends (naked hands  $P= 0.011$ , ethanol  $P< 0.001$ ). In all time points, minimum bites were observed in traditional repellents and it was significantly less than the other groups ( $P< 0.001$ ). The time trend in the number of bites in the other groups was positive and showed minimum number of bites in time zero in all groups. We also found that the concentration of repellents had association with the number of bites. The maximum and minimum numbers of bites were observed with 10% and 40% concentrations respectively in all groups.

**Conclusion:** Essential oils of *Salvia sclarea*, *Lavendula officinalis* and *Myrtus communis* have repellency effect, even with 10% concentration of essential oils.

**Keywords:** Malaria, repellents, *Anopheles stephensi*, essential oil, plants

## Introduction

Using of special lotions and repellent sprays on skin is one of the effective methods to prevent Arthropods biting. However, research to find more effective repellents with minimum side effects is a very attractive research field for scientists with different backgrounds.

As an example, DEET (N, N-diethyl-3-methyl benzamide) is one of the most effective repellents used worldwide for six decades (Katz et al. 2008). However, similar to other

repellent, it might have irritant effects mainly on sensitive skins or other side effects (Antwi et al. 2008, Katz et al. 2008). In addition, the maximum efficacy of these substances varies between one up to twelve hours based on their ingredients and concentrations (Katz et al. 2008). Until now the environmental protection agency's has approved DEET, picaridin, MGK-326, MGK-264, IR 3535, oil of citronella, and oil of lemon eucalyptus as Insect

Repellent (IR) ingredients for application to the skin (Katz et al. 2008).

Based on the above explanation, continuously search is needed to find more potent materials with minimum side effects and longer durability. Among these studies, research on the repellency effect of essential oils and national products of plants has a very unique position.

*Satureja khuzestanica*, *Salvia sclarea*, *Lavandula officinalis* and *Myrtus communis* grow in West of Iran, across Zagros mountains and other parts of the country. Insecticidal and larvicidal effects of different doses of essential oils of *S. khuzestanica* and *M. communis* have been reported (Uehleke and Brinkschulte-Freitas 1979, Traboulsi et al. 2002, Yang et al. 2004, Yi et al. 2006, Cetin et al. 2007, Martinez-Romero et al. 2007, Knio et al. 2008, Amer and Mehlhorn 2009). Other researchers have stated antimicrobial, antifungal and antiparasite effects of mentioned plants and essential oils of two other plants (*L. officinalis* and *S. sclarea*) that grow in Iran (Hayder et al. 2004, Shahidi-Bonjar 2004, Sonboli et al. 2006, Pinto et al. 2006, Ben Arfa et al. 2006, Appendino et al. 2006, Mahdi et al. 2006, Burt et al. 2007, Cristani et al. 2007).

Repellency effect of essential oil of *L. officinalis* has been reported (Nazemi Rafi and Moharamipour 2008). Local people in some districts of Lorestan Province in West of Iran (Zagross mountains), use dried leafs of local plant “*S. sclarea*” inside their pillows. They believe that the plant protect them from biting of insects during night (Ali Salehnia, personal communication).

Although scattered evidence is available about the repellency effects of the mentioned four substances, we did not find any studies that compare their effects using standard methodology. So, based on above explanations we evaluated repellency effect of essential oils of 4 mentioned plants using Standard WHO Repellency Tests.

## Materials and Methods

### Extraction method

*L. officinalis* (Ostocdoos, local name, Family: Lamiaceae) is a decorative plant and is used for decorations of parks and gardens. We bought dried leafs and flowers of mentioned plant from Hamedan City in West of Iran (latitudes 34.8000 and longitudes 48.5167). *M. communis* (Moort, local name, Family: Myrtaceae) was collected around the Khorramabad City in West of Iran (latitudes 33.4841 and longitudes 48.3525). This plant grows in West of Iran. The name, species and family of selected parts of plants were confirmed by academic staffs of Research Center of Agricultural Department, Lorestan Province (Shahla Ahmadi, personal communication).

Leafs and flowers of *L. officinalis* and leafs of *M. communis* were dried and grind. Essential oils of plants were separately extracted by Clevenger (model BP, British Pharmacopoeia, manufacturer Ashke Shisheh Company, Iran and mantle model H610 manufacturer Fater Company, Iran) used hydro distillation method at 60 °C. Duration of extraction was 1 hour for each 0.5cc essential oil. Sodium sulfate was used for dehydration (Stahl-Biskup and Saez 2002, Okoh et al. 2010, Gavahian et al. 2012).

All above procedures were carried out in laboratories of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences. We extracted respectively 3cc, 1.3cc and 0.5cc pure (about 100%) essential oils of 100gr of dried flowers and leaves of *L. officinalis* and dried leaves of *M. communis*.

Essential oils of *S. khuzestanica* (carvacrol, Family: Lamiaceae), and *S. sclarea* (salvia, Family: Lamiaceae) were bought from Khorraman Pharmaceutical Company, Khorramabad, Iran. This company culture *S. khuzestanica* and extracts its essential oil (carvacrol) for medicine marketing. *S. khuzestanica* grows in West of Iran (South of Lorestan and North

of Khuzestan Provinces) and *S. sclarea* grows in West of Iran. The mentioned company extracted respectively 3cc and 0.5cc pure (about 90% to 94%) essential oils of 100 gr of dried leaves of *S. khuzestanica* and *S. sclarea* (Ali Salehnia, personal communication). All essential oils were kept in 4 °C.

### Repellency tests

WHO guidelines for efficacy testing of mosquito repellents for human skin were used (WHO 2009). Separate solutions with 10%, 20% and 40% concentrations of essential oils of plants in 99.6% ethanol were prepared. Traditional repellents (DEET, 50% N, N-diethyl-3-methyl benzamide) and 33% N, N-diethyl-3-methyl benzamide and negative control (pure ethanol, 99.6%) were bought respectively from Nomad, Turnpike lane, London N8 OPX, Medical limited and School of Pharmacy, Shiraz University of Medical Sciences in Iran.

The essential oils, traditional repellents as gold standard repellents (positive controls) and negative controls were rubbed on right and left forearms of four eligible volunteers from elbow to wrist and their hands were protected by gloves to make it unattractive to mosquitoes. The whole forearm exposed in a mosquito cage (50cm× 50cm×50cm) with 50, 2 or 3 days old females of susceptible strain of *An. Stephensi* reared in insectarium, unfed and hungry. Hands were kept inside the cage for 45 seconds and numbers of bites were recorded. This procedure was repeated 3 times. Negative controls (naked hands and hands rubbed with pure ethanol) and positive controls (DEET, 50% N, N-diethyl-3-methyl benzamide and Shiraz 33% N, N-diethyl-3-methyl benzamide) were used in this experiments as well. All tests were repeated every hour up to five hours after the first tests. The used mosquitoes for tests changed in every test (WHO 2009).

Repellency tests were started at 5.30AM and continued to 10.30AM. We used 3 cages of

*An. Stephensi* per day each cage had 50 mosquitoes. Tests were carried out in a half-dark room (27 °C and 70% humidity) near insectary.

The tests were carried out in Bandar Abbas Health Research site affiliated to National Institute of Health, Tehran University of Medical Sciences. This research has been approved by Ethical Committee of Lorestan University of Medical Sciences.

### Statistical Analysis

With respect to the ethical considerations, we minimized the number of humans that take part in the project, and the first author of the paper personally participated in the trial. However, in order to maximize the statistical power of our analysis and according to WHO guidelines for efficacy testing of mosquito repellents for human skin, we repeated each trail three times, with six measurements over 5 hours.

The data were computerized using SPSS version 11.5. The number of bites in each experiment was the main dependent variable. We checked the distribution of the number of bites in each concentration and each group by comparing the 95% confidence intervals of their skewness and kurtosis with their values in a normal distributed variable; since we did not find any significant difference in these analyses, we used parametric tests to compare the number of bits groups and concentrations. We used repeated measure ANOVA to assess the effect of time and also the type of repellents. In addition, the linear trends in the number of bites were computed using regression model. In all statistical analysis, 0.05 was considered as significant level.

### Results

The repellency effects of Nomad (DEET, 50% N, N-diethyl-3-methyl benzamide) and Shiraz (33% N, N-diethyl-3-methyl benzamide) in all tests were comparable up to 5 hours

( $P= 0.571$ ), so we decided to incorporate these two traditional groups to improve the power of statistical tests as gold standard positive control (DTA).

Based on the results of few first tests with essential oil of *S. khuzestanica*, side effects were considerable, mainly the burning, reddening of skin, and irritation for several hours after rubbed on forearms, so essential oil of *S. khuzestanica* was dropped from repellency tests.

Figure 1 shows the marginal means of the number of bites per session (adding together the results of different concentrations based on the results of multivariate analysis) of *An. stephensi* up to 5 hours classified by the type of repellents and control groups. Based on these findings, in negative control groups, the number of bits were comparable ( $P= 0.42$ ) and had decreasing time trends (naked hands  $P= 0.011$ , ethanol  $P< 0.001$ ), started from around 30 bites in time zero, reached to around fourteen in negative control group (naked hands), and six in another negative control group (ethanol, 99.6% ) after five hours.

In all time points, minimum bites were observed in DTA group (less than 2 bites) and it was significantly less than the other groups ( $P< 0.001$ ). The time trend in the number of bites in the other groups was positive and showed minimum number of bites in time zero in all groups (less than 2 bites). However, this trend in *L. officinalis* was more

prominent (the average of bites in time five was 9.8,  $P= 0.001$ ), while the corresponding numbers in *S. sclarea* and *M. communis* were less than four ( $P= 0.04$  and  $P= 0.011$ ) respectively.

The regression coefficients for the effect of time (which shows the average of changes in the number bites between two consecutive measures) were 0.29, 0.40, 0.58, and 0.20 for *S. sclarea*, *M. communis*, *L. officinalis* and DTA (as positive control) respectively. While these coefficients for negative controls (naked hands and pure ethanol) were -2.88 and -3.60.

There were significant difference in the number of bites between negative controls and other repellents ( $P< 0.001$ ). In time five, the difference between negative controls and *M. communis* was significant ( $P= 0.04$ ). However, the other groups had significant difference only with negative control groups in time five ( $P< 0.05$ ).

We also found that the concentration of repellents had association with the number of bites (Fig. 2). The maximum and minimum numbers of bites were observed with 10% and 40% concentrations respectively in all groups. In *Salvia* and *M. communis* groups, only the difference between 10% and 40% concentrations were significant ( $P= 0.032$  and  $P= 0.015$  respectively). While in *L. officinalis* even the difference between the minimum and maximum concentrations had borderline  $P$ -value (0.063).

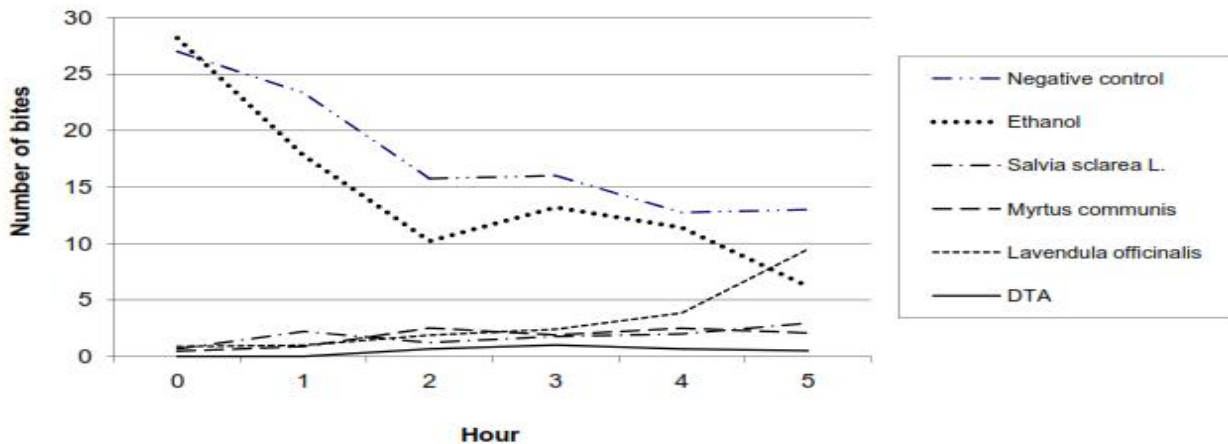


Fig. 1. The time trends in the number of bites per session classified by the type of repellents and control groups

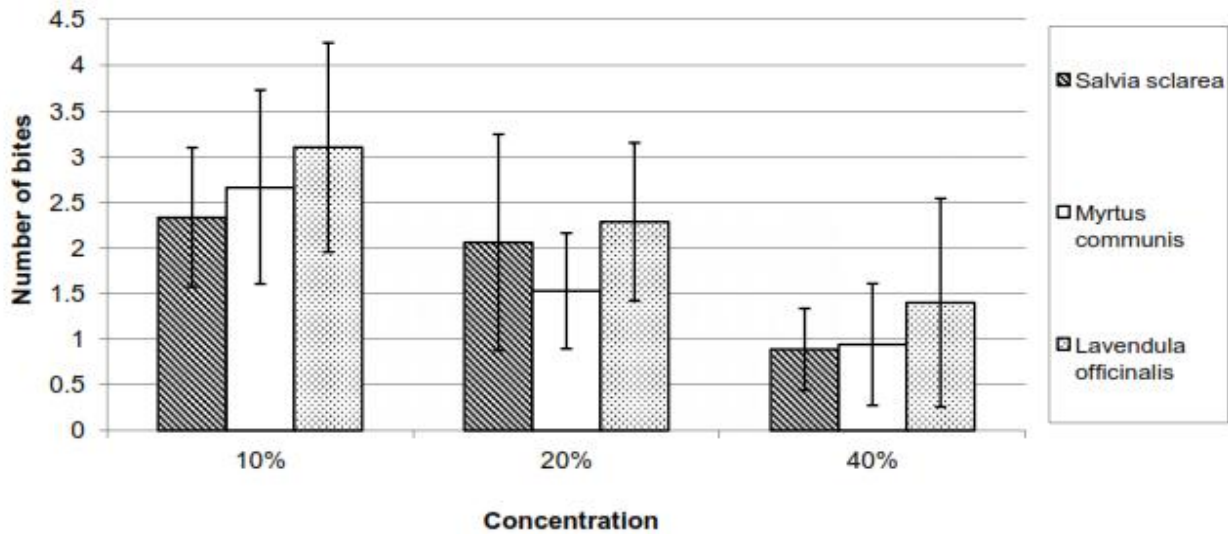


Fig. 2. Comparison between numbers of bites in different concentrations classified by the type of repellents

## Discussion

Our results showed that the essential oils of *M. communis*, *L. officinalis* and *S. sclarea* have repellency effects for *An. stephensi*. Although by time, their repellency effect decreased but even after five hours, their effects were more than the effects in control groups.

We found a sharp decreasing trend in the number of bites in negative control groups. This decreasing trend might be explained by this fact since we started our experiments very early morning and continued up to 10.30AM. However, this decreasing trend was not observed in the other groups which might be due to the attenuation in the repellency effects of these substances.

By time, the repellency effect of essential oils of plants was reduced but even after five hours still they had significant effect more or less comparable with the effect of DTA which is a known and standard repellent.

Although the maximum effect was observed in samples with 40% concentration, even the effects of samples with 10% concentration were significant and comparable with higher concentrations. This means that for further studies 10% concentration can be used to as-

sess the repellency effects of these substances or even similar ones. These findings are consistent with a similar study carried out by Tavassoli et al. (2011). Although their methods was different with our methods, they reported that repellency efficiency of 50% concentration of essential oil of *M. communis* (myrtle) was moderate and lower than DEET.

Other researchers have studied repellency effects of other plants in Iran. Oshagi et al. (2003) evaluated repellency effect of extracts and essential oils of *Citrus limon* Burm, (lemon) and *Melissa officinalis*, (Balm) using *An. stephensi* in laboratory on animal and human in comparison with DEET. They showed significant differences between oils and extracts ( $P < 0.05$ ) and reported that difference between DEET and lemon oil was not significant. They recommended lemon essential oil as an alternative to DEET. In another study Vatandoost and Hanafi-Bojd (2008) compared the repellency effect of 3 topical repellents (permethrin, DEET and neem tree extract) against *An. stephensi* in Iran. They reported that: "Major heterogeneity of response was observed using DEET. Although neem was

the least effective agent, extracts of locally produced neem oil offer a promising repellent against mosquito biting”.

Repellency effect of eucalyptus based plant has been shown by researchers in different trials in the Bolivian Amazon (Moore et al. 2002), China (Yang and Ma 2005) and Guinea Bissau (Palsson and Jaenson 1999). Ansari et al. (2005) reported that Pine oil (*Pinus longifolia*, Family: Pinaceae) which is used by people in India for protection against mosquito bites provide 100% protection against *An. culicifacies* up to 11 hours and 97% protection against *Culex quinquefasciatus* for nine hours. They recommended that aromatic oil of Citronella and Lemon eucalyptus may be promoted as a repellent. Two investigators reported repellency effect of neem tree extract from trials in Iran (Vatandoost and Hanafi-Bojd 2008) and Bolivian Amazon (Moore et al. 2002).

Assessment of the repellency effects of essential oils of plants is a very attractive field for entomologists around the world (Palsson and Jaenson 1999, Curtis et al. 2002, Oshaghi et al. 2003, Seyoum et al. 2003, Omolo et al. 2004, Innocent et al. 2008, Vatandoost and Hanafi-Bojd 2008). Many researchers have carried out trials on repellency effect of different plants. In a study 41 plant extracts and 11 oil mixtures were evaluated using *Aedes aegypti*, *An. stephensi* and *Culex quinquefasciatus* on human volunteers. They reported Litsea (*Litsea cubeba*), Cajeput (*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepeta cataria*) that have been most effective oils of plants with 100% repellency against three mentioned mosquitoes and up to 8 hours protection time (Amer and Mehlhorn 2006). Investigators reported repellency effect of six plant species that grow in the Kenyan coast (Odalo et al. 2005), three plant species that grow in Western Kenya (Seyoum et al. 2003) and six plant species that grow in the Kenya (Odalo et al. 2005).

Their insecticidal and larvicidal effects have

been documented, for example, the essential oils of *S. khuzestanica* and *M. communis* had significant larvicidal and insecticidal effects (Traboulsi et al. 2002, Amer and Mehlhorn 2006, Yic G et al. 2006, Cetin et al. 2007, Martinez-Romero et al. 2007). Nonetheless, their toxic effects might be a point of concern particularly after high dose of oral consumption (Uehleke and Brinkschulte-Freitas 1979).

Jaenson et al. (2006) reported that: *Lavandula angustifolia* (lavender oil) and *Pelargonium graveolens* (geranium oil) have 100% repellencies against host-seeking nymphs of *Ixodes ricinus* when diluted to 30% in 1.2 propanediol. His results were similar to our results with *L. officinalis*. A study that was carried out in Iran by Nazemi and Moharamipour (2008), showed the repellency effect of *L. officinalis* on red flour beetle, *Tribolium castaneum*. They showed that after five hours the repellency effect was still considerable and significant, also they showed positive association between the concentration and repellency effect. These results were consistent with our results in the present study.

## Conclusion

We found that the different concentrations (40%, 20% and 10%) of essential oils of *S. sclarea*, *L. officinalis* and *M. communis* had considerable repellency effect on *An. Stephensi* even with low concentration and after five hours. These findings might open windows for formulating new repellent with less side effect and longer durability.

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