



# Repellent Efficacy of *Eucalyptus globulus* and *Syzygium aromaticum* Essential Oils against Malaria Vector, *Anopheles stephensi* (Diptera: Culicidae)

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## Abstract

**Background:** Mosquito species are highly considering as disease transmission as well as nuisance insects. One of the principal strategy to protect human from the mosquito bites is repellent agents. This study aimed to assess repellency of two organic essential oils, *Eucalyptus globulus* and *Syzygium aromaticum* from bites of malaria vector, *Anopheles stephensi*.

**Methods:** The study was conducted in 2019-2020. The components of essential oils of *E. globulus* and *S. aromaticum* was determined using gas chromatography/mass spectrometry. The unfed female mosquitoes aged 2-5 d old were used in all experiments. In vivo Klun and Debboun module bioassays were utilized on human-volunteer skin. The essential oils at serial concentrations were used to find repellent efficacy against *Anopheles* landings and bites. To find the synergistic effect, four combinations of the essential oils were tested.

**Results:** The main composition of *E. globulus* essential oil was 1,8-Cineol (78.20%), whereas that of *S. aromaticum* essential oil was 2-methoxy-3-(2-propenyl) (77.04%). Based on minimum effective dose ( $\leq 1\%$  biting), 10% (v/v) of *E. globulus* showed high landing repellency (77.78%), whereas minimum effective dose of *S. aromaticum* at concentration of 1% had high landing repellency (88.89%). Among four combinations, the ratio of 1:1 of *E. globulus* (10%):*S. aromaticum* (1%) showed the most landing repellency (94.44%).

**Conclusion:** The combinations of two essential oils had the most potential repellency effect against landing of mosquitoes. As essential oils are eco-friendly with less irritation for human skin, *E. globulus* and *S. aromaticum* essential oils are recommended as effective and safe mosquito repellents.

**Keywords:** Repellent activity; *Anopheles stephensi*; *Eucalyptus globulus*; *Syzygium aromaticum*

## Introduction

Mosquito vectors play vital role in transmission of fatal diseases i.e., malaria, dengue, chikungunya,

zika, filariasis and various forms of encephalitis (1, 2). Synthetic insecticides are the first line of action



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due to their quick action, but their continuous use may lead to the development of resistance and adverse effect on environment. Therefore, enormous efforts have been made to develop effective repellents and/or insecticides against medically important insects (3).

Repellent agents are generally defined as material that prevent arthropods from landing or biting on human skin. One of the synthetic repellents is DEET (N, N-diethyl-3methylbenzamide). Mechanism of repellency activity of DEET is blocking the olfactory (odorant) receptors (ORx), which are mostly on antenna and maxillary palps of the insects and exposed to external environment. The ORx receptors are sensitive to detect l-octen-3-ol, the volatile odor, which found in human sweat. However, DEET is not able to block the carbon dioxide detection ability of insect's found in human breath (4, 5).

DEET has been associated with some concerns as environmental risks and side effects as neurotoxicity, irritation and allergy in human, especially in children as well as in lactate and pregnant women (6, 7). Accordingly, there is a worldwide necessity to explore eco-friendly, biodegradable and safe alternative repellents. The use of plant materials (extracts or essential oils) as repellent, has been used for thousands of years. Herbal materials, applied to the skin, cloths or as fumigants to protect human from mosquitos' nuisance. Furthermore, the plant extracts are more safe and easy to stock in houses in comparison with chemicals (4, 8). Therefore, some of plant materials which display respectable repellent properties have been considered as alternatives to synthetic repellents (8).

Herbal repellent property evaluation and developments in methods of formulations to reduce the evaporation speed, cause increasing the longevity of volatile components, which are responsible for repellency activity (4). For instance in a comparative study of repellency effect of 38 essential oils, include *Eucalyptus globulus* and *Syzygium aromaticum*, in three concentrations (10%, 50% and undiluted), 32 of them prevented mosquito bites in undiluted form (9). Some of essential oils include *E. globulus* and *S. aromaticum* found that an effective time of

repellency strongly depended on the concentrations, experiment designs and mosquito species (4).

Up to 90% of Eucalyptus essential oil contains aromatic component eucalyptol or 1,8-cineole. Eucalyptol is a water insoluble compound but soluble in ether, ethanol, and chloroform. It is reported as an insect repellent, insecticide, mosquito larvicide, ovipositional repellent as well as inhibitor acetylcholinesterase activity (10).

Clove components consisted of 70%-90% Eugenol which cause clove aroma (11). This phenolic molecule is recognized as a relatively strong and moderately durable mosquito repellent (4). The concentrated form of eugenol or with cinnamon and clove oil were used as a mosquito repellent. In addition, it is reported as an effective insecticide eugenol and mixtures of eugenol with alpha-terpineol and cinnamic alcohol were used against American cockroaches (*Periplaneta americana*) and German cockroaches (*Blattella germanica*).

Eugenol is also considered to be extremely volatile and degradable in air, soil, and water, including through microbial activity (12).

However, both above component have been relatively safe for mammals (13), median lethal doses (LD 50) of the essential oils from *E. globulus* and *S. aromaticum* on albino rats (oral) were 2,334.4 and 3,597.5 mg/kg b.w., respectively.

In the current study, we aimed to provide more information about repellency activity of *E. globulus* and *S. aromaticum* essential oils in different concentrations and combinations against *An. stephensi*, as well as their natural components, for future investigations to achieve the best formulation of *E. globulus* and *S. aromaticum* essential oils, as an effective and safe mosquito repellent on human skin.

## Materials and Methods

### Mosquitoes

Adult females of *An. stephensi* used in this study, were from the laboratory colonies reared and maintained in an insectary, under the conditions at  $28 \pm 2$  °C,  $60 \pm 10\%$  relative humidity (RH) and 12:12 (L:D) h photoperiod. The mosquitoes were

fed with 10% sugar solution and the females were offered guinea pig blood twice in a week. Non-blood fed 2-5 d old females kept starve for 4-6 h prior to use for bioassay tests. Tests were conducted in laboratory test room with the help of Klun and Debboun test module.

### Essential Oils

*E. globulus* essential oil was purchased from Keshto-Sanate Golkaran Kashan Co. (Batch No: 97-4). The buds of clove (*S. aromaticum*) were procured from a local market, the buds were grained by a grinder and then hydro-distilled for 6 h in a Clevenger type apparatus. The oil was then dried over anhydrous sodium sulphate and kept in dark glass at 4-5 °C in a refrigerator until analysis. The density of *E. globulus* and *S. aromaticum* essential oils were estimated based on weight per volume (mg/ml).

### Analysis of Essential Oils

Gas chromatography/mass spectrometry (GC/MS) analyses were performed on *S. aromaticum* and *E. globulus* essential oils. A gas chromatograph 7890B (Agilent Technologies) equipped with a 30 m DB-5 capillary column (0.25 mm inner diameter, 0.25 µm film thickness) was used in combination with a mass spectrometer 5977A (Agilent Technologies, electron ionization detector). All injections were performed in split mode with auto sampler. A 10-µl syringe was used and the injection volume was 1 µl. The injector temperature was set at 280 °C and the flow rate was maintained at 1.0 ml/min using helium as the carrier gas. The initial oven temperature was set at 50 °C for 5 min and ramped at 10 °C/min to 280 °C. The ion source and interface temperature were 230 °C and 300 °C, respectively. The Mass Selective Detector (MSD) was used in the electron impact (EI) full scan monitoring mode with a solvent delay time of 5 min.

### K&D module bioassay

The mosquito repellency tests were conducted using Klun & Debboun (K&D) test module which made up of plexiglas for quantitative evaluation of biting and landing deterrence, with little modification (14, 15). The device consists of 4 cells and each cell has a circular (1cm diameter) access whole for introduction of mosquitoes and a rectangular window (3cm×4cm) with a sliding door at the bottom. The rectangular window can be opened by sliding the door, when desired. Twenty-five microliter of test solution was applied on 3cm×4cm marked area of skin (2.1 µl/cm<sup>2</sup>) and allowed to dry. Then 5 unfed female mosquitoes at age of 2 to 5 days old placed into each alternate cells of the K&D apparatus slightly modified. The bottom shape of K&D module apparatus was slightly concave conformed to the curvature of non-smoking volunteers forearm (14) (Fig. 1). In order to find Minimum effective dose (MED≤1% biting) on mosquito biting, different concentrations and combinations of essential oils in deionized water were prepared and tested as recommended (16). The range of concentrations were 3%, 5% and 10% for *E. globulus* and 0.5%, 1%, 3% and 5% for *S. aromaticum*. To determine the synergistic effects, 6 different combinations of both oils were prepared according to the minimum effective concentrations, at ratios: 0.5:0.5, 0.5:1, 1:0.5, 1:1, 1:2 and 2:1 (v/v). As positive control, we used DEET 40% purchased from Reyhan Naghsh Jahan, Isfahan Pharmaceutical Co. Distilled water was used as negative control. Furthermore, *E. globulus* 10%, *S. aromaticum* 1% and *E. globulus* 10% + *S. aromaticum* 1% repellency activities were followed since 2 h post application. The number of mosquitoes landing and biting on treated skin in each cell within 3 min exposure was recorded. The test was conducted with four replications for each concentration or combination of both oils.



**Fig. 1:** K&D module apparatus with slightly modification at four cells and bottom concave conformed to the curvature of a human forearm

### Data Analysis

The percentage of repellency was calculated by comparing the landing number for treated skin with the controls. The percentage of repellency was calculated at the end of every test by using the formula as described by Sharma and Ansari (17).

$$\% \text{ Repellency} = \frac{C - T}{C} \times 100$$

Where C is the total number of mosquitoes landing and/or biting at the control area and T is the total number of mosquitoes landing and/or biting at a treated area.

### Statistical Analysis

Data generated in repellent activity experiments using K&D module were expressed as mean % repellency  $\pm$  SD (Standard deviation). The data were then subjected to analysis by adopting analysis of variance (ANOVA) followed by Post Hoc test of LSD for multiple comparisons. The significant differences between the means were analyzed by one-way analysis of variance (ANOVA) and Post

Hoc and Dunnett t-test (2-sided) tests by a probability value (*P*-value) of less than 0.05 was considered to be significantly different. All Statistical analysis was performed by means of statistical software SPSS ver. 21.0.

### Ethical approval

The study was approved by the Ethical Committee of Tehran University of Medical Sciences, Iran (IR.TUMS.VCR.REC.1397.224)

## Results

### Essential Oil Characterization

Twenty chemical components of eucalyptus oil were detected. The main compositions were 1,8-Cineol (78.20%) followed by Alpha-pinene (10.48%) and 2.24% of Gamma-terpinene (Table 1). In addition, eight chemical components was obtained from *S. aromaticum* essential oil. The main components were 2-methoxy-3-(2-propenyl) (77.04%), Trans-Caryophyllene (11.99%), 2-methoxy-4-(2-propenyl) (6.83%). The density of *E. globulus* and *S. aromaticum* essential oils were estimated as 0.957g/ml and 1.061g/ml respectively.

**Table 1:** Components of *Eucalyptus globulus* essential oil from GC/MS analysis

| No. | Compounds                | Rt* (min) | Compositions (%) |
|-----|--------------------------|-----------|------------------|
| 1   | Alpha pinene             | 7.132     | 10.48            |
| 2   | Beta pinene              | 7.690     | 0.46             |
| 3   | 2-Beta Pinene            | 7.844     | 0.33             |
| 4   | Alpha Phellandrene       | 8.008     | 0.18             |
| 5   | 1,8-Cineole              | 8.364     | 78.20            |
| 6   | Gamma terpinene          | 8.561     | 2.24             |
| 7   | Alpha terpinolene        | 8.826     | 0.51             |
| 8   | Butanoic acid            | 8.931     | 0.34             |
| 9   | Bicyclo heptan-3-ol      | 9.277     | 0.99             |
| 10  | Pinocarvone              | 9.455     | 0.35             |
| 11  | 3-Cyclohexan-1-ol        | 9.547     | 1.14             |
| 12  | 3-Cyclohexene-1-methanol | 9.639     | 1.33             |
| 13  | Trans-Carveol            | 9.890     | 0.33             |
| 14  | Cyclohexanol             | 10.622    | 0.42             |
| 15  | Camphene                 | 11.209    | 0.34             |
| 16  | 1H-Cycloprop[e]azulene   | 11.839    | 0.17             |
| 17  | Alloaromadendrene        | 11.926    | 0.47             |
| 18  | Epiglobulol              | 11.966    | 1.02             |
| 19  | spathulenol              | 12.010    | 0.47             |
| 20  | Azulene (-Globulol)      | 12.681    | 0.24             |
|     | Total                    |           | 100              |

\*Retention time

**K&D module bioassay**

The MFD of *E. globulus*, at concentration of 10% which was significantly effective comparing with 5% and 7% but no significantly difference was observed with repellency effect of DEET40% ( $P=0.05$ ). The minimum effective dose for *S. aromaticum* was estimated at 1% which was significantly deferent to concentration of 0.5% however not significantly deferent to concentrations of 3%, 5% and DEET40% ( $P=0.05$ ) (Table 2). Among the different combinations, based on minimum effective dose, the ratio of *E. globulus*10%: *S. aromaticum* 1% (1:1) showed the highest repellency activity as 94.44%, which was significantly different to concentrations of 5% and 7% of *E. globulus*, and concentration of 0.5% of *S. aromaticum* and ratio of

*E. globulus* 5%: *S. aromaticum* 0.5% (0.5:0.5), but not significantly deferent was observed between repellency effect of mixture of *E. globulus* 10%, *S. aromaticum* 1%, at ratios of 1:2 and 2:1 with DEET40% ( $P=0.05$ ) (Table 2).

All three repellent formulations tested significantly reduced the mean number of mosquito bites on treated skin in comparing with untreated forearms skin, however all formulations significantly reduced the number of mosquito landings except *S. aromaticum* at concentration of 0.5% (Table 2,  $P=0.05$ ). The results of repellency activities of *E. globulus* 10%, *S. aromaticum* 1% and *E. globulus* 10% + *S. aromaticum* 1% after 2 h post application are represented in Fig. 2.

**Table 2:** Repellency activity (Mean±SD)% of different concentrations and ratios of *E. globulus* and *S. aromaticum* versus negative and positive control groups over 3 min against *An. stephensi* mosquitoes in laboratory K&D test, 4 replications and total no. of 20 mosquitoes for each test

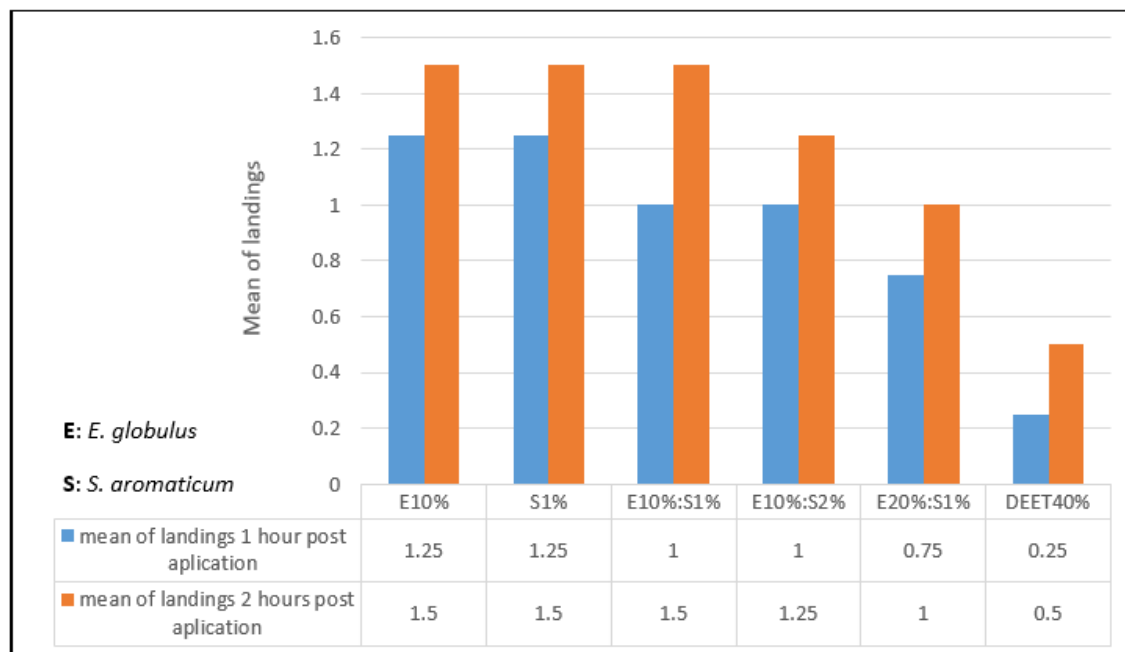
| Treatments  | Concentrations or ratios* | Mean no. of landings | Mean no. of bitings | Passed or Failed** | % repellency of landing ± SD | P-value |
|---|---------------------------|----------------------|---------------------|--------------------|------------------------------|---------|
| <i>E. globulus</i>                                      | 5%                        | 1.5                  | 0.75                | F                  | 66.67±28.69                  | 0.348   |
|   | 7%                        | 0.75                 | 0.5                 | F                  | 83.33±21.28                  | 0.965   |
|   | 10%                       | 1                    | 0                   | P                  | 77.78±18.14 <sup>b</sup>     | 0.798   |
| <i>S. aromaticum</i>                                    | 5%                        | 1                    | 0                   | P                  | 77.78±18.14 <sup>b</sup>     | 0.568   |
|   | 3%                        | 1                    | 0                   | P                  | 77.78±18.14 <sup>b</sup>     | 0.568   |
|   | 1%                        | 0.5                  | 0                   | P                  | 88.89±12.83 <sup>b</sup>     | 1.000   |
|   | 0.5%                      | 4.5                  | 1                   | F                  | 00.00±12.83 <sup>a</sup>     | 0.000   |
| 5% v/v <i>E.globulus</i> + 0.5% v/v <i>S.aromaticum</i> | 0.5:0.5                   | 2.25                 | 0.5                 | F                  | 50.00±11.11 <sup>a</sup>     | 0.001   |
| 10% v/v <i>E.globulus</i> + 1% v/v <i>S.aromaticum</i>  | 1:1                       | 0.25                 | 0                   | P                  | 94.44±11.11 <sup>b</sup>     | 0.914   |
| 10% v/v <i>E.globulus</i> + 2% v/v <i>S.aromaticum</i>  | 1:2                       | 0.5                  | 0                   | P                  | 88.89±12.83 <sup>b</sup>     | 1.000   |
| 20% v/v <i>E.globulus</i> + 1% v/v <i>S.aromaticum</i>  | 2:1                       | 0.5                  | 0                   | P                  | 88.89±12.83 <sup>b</sup>     | 1.000   |
| DEET 40% (positive control)                             | 40%                       | 0.5                  | 0                   | P                  | 88.89±12.83                  | 1.000   |
| Distilled Water (negative control)                      | -                         | 4.5                  | 2.5                 | F                  | 00.00±12.83 <sup>a</sup>     | 0.000   |

\* Ratios were prepared based on the minimum effective dose.

\*\* Based on calculated Minimum Effective Dose (MED) for 20 mosquitoes (≤0.2biting)

a: significant with positive control (DEET 40%) according to Dunnet t test (P≤0.05)

b: non-significant with positive control according to Post hoc LSD test (P≥0.05)



**Fig. 2:** Comparison of mosquito landings on treated skin after one and two h, using K&D module bioassay test

## Discussion

### Essential Oil Characterization

In present study, two main components in the essential oil of *E. globulus* were 1,8-Cineol (78.20%), Alpha-pinene (10.48%). The chemical profiles of the essential oil of *E. globulus* in this study were similar to those collected from India, Italy, Montenegro and Spain (18). Two major compounds of *E. globulus* essential oil have been identified from Indian eucalyptus as 1,8-Cineol (68.8%) and Alpha-pinene (28%), from Italy as 1,8-Cineol (84.9%), Alpha-pinene (5.6%), from Montenegro as 1,8-Cineol (85.5%), Alpha-pinene (7.2%), from Spain as 1,8-Cineol (63.8%), Alpha-pinene (16.1%) (18). However, the second major component of *E. globulus* essential oil from Algeria, China and India were different with the ingredient of our

eucalyptus. For example, the essential oil of *E. globulus* harvested from Algeria mainly contained 1,8-Cineol (78.45%), o-cymene (2.18%) (19). The majority components of eucalyptus essential oil from China contained 1,8 Cineol (94.30%), 2-N-PropylPridine (1.12%), Gamma terpinene (1.10%) and m-cymene (1.04%) (20), while the Indian's eucalyptus mainly contained 1,8 Cineol (66.28%) and cis-ocymen (21.33%) (21).

Three principal components of the essential oil of *S. aromaticum* were 2-methoxy-3-(2-propenyl) (77.043%), Trans-Caryophyllene (1.987%) and 2-methoxy-4-(2-propenyl) (6.829%) (Table 3). 2-methoxy-3-(2-propenyl) (Eugenol) is a volatile phenolic constituent of clove essential oil obtained from *S. aromaticum* buds and leaves, mainly harvested in Indonesia, India and Madagascar. Eugenol is the main extracted constituent (70%-90%) of cloves and responsible for clove aroma (11).

**Table 3:** Components of *Syzygium aromaticum* essential oil from GC/MS analysis

| No. | Compounds                        | Rt* (min) | Compositions (%) |
|-----|----------------------------------|-----------|------------------|
| 1   | Phenol, 2-methoxy-3-(2-propenyl) | 10.721    | 77.04            |
| 2   | Alpha.-Copaene                   | 10.836    | 0.81             |
| 3   | Trans-Caryophyllene              | 11.113    | 11.99            |
| 4   | Alpha.-Caryophyllene             | 11.290    | 1.40             |
| 5   | Delta.-Cadinene                  | 11.365    | 0.29             |
| 6   | Phenol, 2-methoxy-4-(2-propenyl) | 11.559    | 6.83             |
| 7   | Beta-Cadinene                    | 11.599    | 1.21             |
| 8   | Caryophyllene oxide              | 11.971    | 0.43             |
|     | Total                            |           | 100              |

\*Retention time

In this study, the chemical profiles and Eugenol derives of the essential oil of *S. aromaticum* were different to those collected from Indonesia and Madagascar. The major compounds of *S. aromaticum* essential oil from Indonesia was reported as m-Eugenol (69.44%), Eugenol acetate (10.79%), Tyranton (7.78%) and Caryophyllene (6.80%) (22). While the main composition of *S. aromaticum* essential oil form Madagsacar were Eugenol (82.6%), Beta-Caryophyllene (7.2%) and Eugenol acetate (6%) (23).

The environmental factors include temperature, rainfall, humidity and solar radiation as well as the amount of soil macro- and micronutrients are well described as being able to influence the production of metabolites. Thus, plants under conditions of stress induced by climate factors may change the production of different metabolite classes. The availability of water is a known factor related to the variation in the production of metabolites in plants (24).

### Bioassay Repellency Tests

The results of K&D bioassay repellency tests indicate that different combinations of *E. globulus* and *S. aromaticum* at ratios of 1:2 showed higher repellency percentage of  $94.44 \pm 11.11\%$ , comparing with individual compounds of *E. globulus* 10% (repellency percentage of  $77.78\% \pm 18.14$ ) and *S. aromaticum* 1% (repellency percentage of  $88.89\% \pm 12.83$ ). The mixture of both essential oils enhance the repellency activity against the mosquitoes.

Different combinations of five essential oils were evaluated against female *Aedes albopictus* mosquito using K&D module (8). Repellency activity of ten essential oils was examined including *E. citriodora* against *Ae. aegypti* using K&D module (25). Using a 6-celled in vitro K&D module bioassay system developed by for quantitative evaluation of biting deterrence activity of the carrot seed essential oil, fractions, and carotol against *Ae. aegypti* and *An. quadrimaculatus* (16).

Shooshtari et al, compared repellency effect of the extracts and oils of *Melissa officinalis*, *Rosmarinus officinalis*, *Lavandula officinalis*, *Citrus limonum*, *E. globulus*, *Ocimum basilicum* as well as DEET against *A. stephensi* in a laboratory condition on guinea pig. However, *L. officinalis* and *E. globulus* oils have been reported as the most effective repellents. The repellency effect of eucalyptus oil at a concentration of 3% was 97.15% against *A. stephensi* (26). Though in our study, the concentration of 10% *E. globulus* showed 77.78% repellency against the mosquito on human skin. This dissimilarity results could be due to using different bait, experiment design and the components *E. globulus* essential oils.

Furthermore, Singh et al., studied on the repellent potential of *S. aromaticum* extracted with acetone, hexane and ethyl acetate was screened under laboratory conditions against *A. stephensi*. They used arm in cage bioassay test and their results showed that the hexane extract of clove was more effective for repellency of the mosquito at 2.5%, 5% and 10% concentrations (27). In our study, MED of clove essential oil was at concentration of 1% which may indicate that the difference in results

may arise from using dissimilar method for bioassay test used, extraction methods as well as components of the oils.

The mosquito repellent activities of *Azadirachta indica*, *Vitex negundo*, *Ocimum sanctum*, *Cymbopogon nardus*, *E. globulus* and *S. aromaticum* were also determined. The extract/essential oil of those plants at ratio of 10% (v/v%) were tested for mosquito repellent activity using arm-in-cage method. The gel and spray forms at concentration of 16% (v/v) were prepared, and showed 100% mosquito repellency. While our results showed 94.44% repellency for combination of 10% *E. globulus* and 1% of *S. aromaticum* (28). The different results may be related to different formulation of essential oils as well as different bioassay method.

Organic synergists may increase the repellency effect of essential oils by reducing evaporation rate of their volatile components. Repellency of *Curcuma longa*, *C. aurantium* and *E. globulus* in different formulations were examined against *Aedes aegypti* and *An. dirus*, showed that increasing 5% of vanillin (as fixative) can increase the protection time of all three essential oils. *E. globulus* essential oils were affected much more by vanillin treatment against *Ae. aegypti* and *An. dirus* than other essential oils (29). In our study, adding 1% *S. aromaticum* to 10% *E. globulus*, increased the repellent efficacy from 77.78% up to 94.44% against *An. stephensi*. Our results also showed that the fixative effect of main components of *S. aromaticum* (as Eugenol) helps to reduce the evaporation rate of aromatic components of *E. globulus* essential oil and extend the repellency of *E. globulus* against *An. stephensi*. which confirms the state of Baker and Grant: "a number of mosquito repellents use eugenol as a component, either in concentrated form or with cinnamon and clove oils" (12).

### Conclusion

Both essential oils exhibited convenient repellency effect against the malaria vector, *An. stephensi*. We also found that the mixture of 10% *E. globulus* and 1% *S. aromaticum* has better efficacy comparing



with other mixture ratio as well as individual essential oil. Therefore, this mixture of both essential oils could be a potential candidate as mosquito repellent. Nevertheless, an economic evaluation should be determined for relative cost-effectiveness when this bio-repellent will be introduced as a self-protection agent against mosquito bites.

## Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

## Conflict of interest

The authors declare that there is no conflict of interest.

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