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Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephensi*

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ABSTRACT

Objective: To determine phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against *Anopheles stephensi*. **Methods:** The chemical compositions of the leaf essential oils were analyzed using gas chromatography/mass spectrometry (GC/MS). The larvicidal activity of essential oils and extract of leaf were tested against 4th instar larvae of laboratory-reared *Anopheles stephensi*. **Results:** GC/MS analyses identified the presence of 28 compounds corresponding to 99.60% of the total oil. The main constituents in the leaf essential oil were 1,8-cineole (69.46%), γ -Terpinene (15.10%), α -Pinene (5.47%) and Globulol (2%). The leaf extract and volatile oil exerted significant larvicidal activity with LC₅₀ values of 89.85 and 397.75 ppm, respectively. Clear dose-response relationships were established with the highest dose of 320 ppm essential oil extract resulted almost 100% mortality in the population. **Conclusions:** The larvicidal properties suggest that the essential oil of plant is a potential source of valuable larvicidal compounds against malaria vector and can be used as an alternative to synthetic insecticides.

1. Introduction

Mosquitoes are considered as vectors of malaria, dengue fever, yellow fever, filariasis, and several other diseases. In addition they are among of the most annoying pests in the world. They comprise a biting problem which can cause allergic responses on humans. More comprehensive efforts have been made to control mosquitoes than any other medically important insects. Malaria remains a major public health problem in southern part of the country which comprises about 80% of all malaria cases in the country. In this part of the country there are six anopheline mosquitoes as malaria vectors including *Anopheles stephensi* (*An. stephensi*)[1–5], *Anopheles culicifacies* (*An. culicifacies*)[6], *Anopheles dthali* (*An. dthali*), *Anopheles fluviatilis* (*An. fluviatilis*)[7–11], *Anopheles superpictus* (*An. superpictus*), and *Anopheles pulcherrimus* (*An. pulcherrimus*)[12–15]. *Anopheles*

sahacrovi (*An. saharovi*)[16,17] and *Anopheles maculipennis* (*An. maculipennis*)[18,19] are considered as malaria vector in northern part of the country.

Chemical control methods have been applied against either the immature or the adult of malaria vectors[20–23]. Chemical larvicides are the most important parts of such programme. Mosquito control, using chemical larvicides have been performed during the fight against malaria in Iran and still are considered as an important part of vector control. Temephos, an organophosphate compound which is recommended as the most appropriate larvicide for the control of *Anopheles*[24] now it is considered as a toxic material to fish and other non-target organisms as well as the environment, and also it causes the increase of insecticide resistance of arthropods. There are several reports indicating resistance of vector to chemical insecticides in Iran. Hence, scientists are looking for alternatives to conventional pesticides which are safe to environment and human health and other non target organisms. There are several reports on the phytochemistry, larvicidal and repellency effect of different plants against malaria vectors[25–30]. The extract of whole leaf and essential oil of certain plants have been investigated, and showed toxic effect against some public health pests[31–35].

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Anopheles mosquitoes as the natural vectors of humane malaria are the subject of such studies. Some of secondary plant metabolites as botanical insecticides have shown larvicidal properties against anopheles and also being eco-friendly^[36].

Eucalyptus is an evergreen non native plant in Iran and now widely cultivated across the country. This genus is originally from Australia belong to the *Myrtaceae* family, *Myrtoideae* subfamily, *Eucalypteae* tribe, and *Eucalyptus* subgenus. It includes more than 700 species while some of them are used in traditional medicine to cure many medical conditions^[37,38]. However, certain species of genus of *Eucalyptus* are widely used in modern medicine. *Eucalyptus camaldulensis* (*E. camaldulensis*) has been used to treat lung diseases and cough in medicines like expectorants^[39]. It has also the antituberculosis, antibacterial and antifungal microbiostatic, disinfectant properties^[40].

Insecticidal properties including larvicidal and mosquito repellent activities of the botanical family Myrtaceae have been investigated. Leaves of the *Eucalyptus* genus in this family have cineol, and show good mosquito repellent activity.

In this study constituents of essential oils using gas chromatography/mass spectrometry (GC/MS) were analyzed and then the efficacy of essential oils and methanol extract from leaf were tested against larval stages of *An. stephensi*.

2. Materials and methods

2.1. Mosquito rearing

The 4th instar larvae of laboratory-reared *An. stephensi* of Bandar Abass strain were used in this study. The colony was established in the insectarium of the Department of Medical Entomology & Vector Control, Tehran University of Medical Sciences and maintained at 27 °C, (70±10)% relative humidity, with a photoperiod of 12 h in light and 12 h in the dark.

2.2. Plant materials

Fresh leaves from 5-year-old tree of *E. camaldulensis* were obtained from herbarium of Faculty of Pharmacology, Tehran University of Medical Sciences. The species were identified and the voucher specimens (84160) were deposited at the herbarium.

2.3. Essential oil isolation

The leaves (100 g) of *E. camaldulensis* were picked out during the morning. The essential oils were isolated by hydrodistillation using a modified Clevenger-type apparatus for 3 h. The obtained oil was dried over anhydrous sodium sulphate, and stored in amber-coloured vials at 5 °C for later investigation.

2.4. Preparation of methanol extract

Dry leaves of plant (200 g) were submitted to percolation with ethanol (1 600 mL, 80%) during 48 h and this procedure was repeated thrice. The extract was then evaporated in a rotary evaporator.

2.5. GC/MS

Water and steam distilled oils of plant were analyzed by a Agilent Technology 5973 mass selective detector connected with a HP 6890 gas chromatograph. The separation was achieved by use of a HP1MS (Fused silica) capillary column (30 m × 0.25 mm; film thickness 0.25 μm) as follows: 40 °C for one min, then to 250 °C at 5 °C per min. The injector temperature and the detector temperature were 250 °C and 230 °C, respectively. Helium rinsing used as the carrier gas (1 mL/min). Mass spectra were taken at 70 eV. Relative percentage amounts were calculated from peak areas using a Shimadzu CR4A chromatopac. GC/MS analysis of the hydrodistilled oil of plant was performed as it was reported previously.

2.6. Identification of the compounds

Retention indices (RI) of the components were calculated using retention times of n-alkans that were injected after the oil at the same chromatographic conditions. The compounds were identified by comparison of mass spectra and RI with authentic samples, published data and data from computer library (Wiley 7n.L).

2.7. Biological study

According to WHO recommendation^[41], 4th instar larvae of *An. stephensi* were exposed to essential oil at concentrations of 10, 20, 40, 80 and 160 ppm and methanol extract in distilled water at 20, 40, 80, 160, 320, 640 and 1 280 ppm for 24 h. Mortality was counted after 24 hours recovery period.

2.8. Statistical analysis

The lethal concentrations (LC₅₀ and LC₉₀) were calculated using Probit analysis^[42]. The percentage of mortality was corrected for control mortality using Abbott's formula and the results were plotted on log/probability paper using the method of Finney (1971). Differences between means were considered significant at *P* < 0.05.

3. Results

3.1. Chemical constituents of essential oils

The constituents of *E. camaldulensis* essential oil are shown in Table 1. The oil was light yellow with a distinct sharp odour in a yielding of 1.8% (w/w) for hydrodistillation. In the essential oil extracted from leaves 28 compounds were identified, corresponding to 99.60% of the total oil. Eight monoterpene hydrocarbons (21.76%), 8 oxygenated monoterpenes (72.29%), 7 sesquiterpene hydrocarbons

(2.37%), and 5 oxygenated sesquiterpenes (3.18%) were identified in the essential oil. The main constituents in the leaf essential oil were 1,8-cineole (69.46%), γ -Terpinene (15.10%), α -Pinene (5.47%), Globulol (2.00%).

Table 1Chemical constituents of leaf essential oils from *E. camaldulensis*.

Compound	RI	Percentage(%)
α -Thujene	899	0.12
α -Pinene	905	5.47
Camphene	922	0.03
β -Pinene	946	0.21
β -Myrcene	957	0.30
1,8-Cineole	998	69.46
β -Ocimene	1 007	0.01
γ -Terpinene	1 028	15.10
Terpinolene	1 057	0.53
1-Terpineol	1 106	0.03
Limonene Oxide(cis)	1 112	0.01
α -Terpineol	1 162	1.29
trans-Carveol	1 189	0.02
Geraniol	1 222	0.13
Geranial	1 240	0.04
α -Terpienyl Acetate	1 327	1.31
α -Gurjunene	1 376	0.34
β -Gurjunene	1 401	0.10
Aromadendrene	1 410	1.72
β -Selinene	1 457	0.06
γ -Cadinene	1 483	0.05
Δ -Cadinene	1 491	0.07
α -Calacorene	1 519	0.03
Epi Globulol	1 541	0.29
Globulol	1 559	2.00
Viridiflorol	1 565	0.61
β -Eudesmol	1 623	0.23
α -Cadinol	1 631	0.05

Table 2Parameters of probit regression line of *An. stephensi* to methanol and essential oil extraction of *E. camaldulensis* at different interval concentrations.

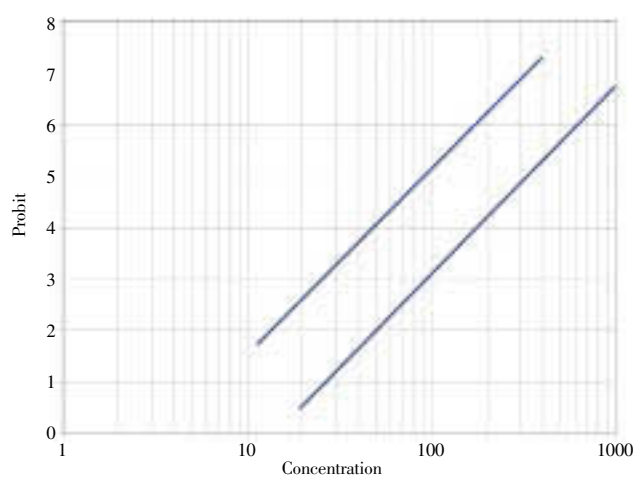
Specimens	A	B \pm SE	LC ₅₀	95% CI	LC ₉₀	95% CI	χ^2	P value
Methanol extract	0.37	1.78 \pm 0.13	397.75	265.98–610.32	3 085.18	1 168.77–6559.68	22.24	<0.05
Essential oil	-1.59	3.37 \pm 0.18	89.85	50.92–129.61	215.26	148.22–422.56	29.50	<0.05

4. Discussion

The tree *Eucalyptus* is cultivated for its oil, pulp, gum, timber medicine values. The oil extract of tree possesses wide range of biological activities such as antimicrobial, fungicidal, insecticidal, insect repellent herbicidal acaricidal and nematicidal[43]. Essential oil, whole plant extract, aqueous extract, leaf extract, acetone extract, ethanolic extract, methanol extract of different plants were used against mosquitoes as adulticide, larvicide, growth

3.2. Mosquito larvicidal activity of essential oil

Table 2 shows the parameters of probit regression line of *An. stephensi* larval susceptibility to methanol and essential oil extraction of *E. camaldulensis* at different interval concentrations. Moreover, probit regression lines for two extracts are shown in Figure 1. There was no significant difference between the essential oils and methanolic extract in this study. Essential oil of *E. camaldulensis* at 320 ppm and 1 280 ppm induced almost 100% larval mortality of *An. stephensi* in 24 hours. From a comparison of the LC₅₀ and LC₉₀ values of essential oil and methanolic extract of *E. camaldulensis* against 4th instar larvae of *An. stephensi* exhibited that essential oil showed an appropriate toxicity against *An. stephensi* larvae. The LC₅₀ values for essential oil and methanolic extract were 50.92–129.61 and 265.98–610.32 ppm, respectively, with corresponding LC₉₀ values of 148.22–422.56 and 1 168.77–6 559.68 ppm, respectively. Control treatments (ethanol and methanol solutions) had no effect on the larvae.

**Figure 1.** Probit regression lines of *An. stephensi* exposed to different interval concentrations of *E. camaldulensis* extraction.

regulator, repellent, anti-popuational and oviposition inhibitor worldwide. According to GC-MS analyze the major constituent of the leaf essential oil is 1,8-cineole. This species has relatively low sesquiterpene contents, as also noted by several studies[44].

The leaf essential oil from *E. camaldulensis* has excellent inhibitory effect against *An. stephensi* larvae.

The essential oil was tested against mature and immature mosquito vector *An. stephensi*. The results showed 100% mortality at 160 ppm[36]. The larvicidal activity of essential oil and pine resin essential oil (turpentine) and their major components (α - and β -pinene and 1,8-cineole) were

determined against *Aedes aegypti*. Larvicidal activity of the essential oil components showed that α - and β -pinene present low LC₅₀ values (15.4 and 12.1 ppm, respectively), whereas pure 1,8-cineole showed an LC₅₀ of 57.2 ppm. There are several report on the repellency of *Eucalyptus* plants against mosquitoes^[45]. Both seed and leaf extract of *E. globulus* against *Culex pipiens* display 100% and 80 % mortality at 1 000 ppm^[46].

Our findings suggested that the use of essential oil from *E. camaldulensis* in insect control is an alternative pest control method for replacement of some chemical compounds on the environment. Although further research is recommended for plant extract formulation under the field condition for understanding the efficacy of plant for vector borne disease control. Additionally *Eucalyptus* trees can be used for drying marshy areas and other plots of land with a high water table. Species that grow rapidly and use a lot of water are particularly suitable. The trees dry the land by allowing water to evaporate through their leaves.

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Conflict of interest statement

We declare that we have no conflict of interest.

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