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Larvicidial Effects of essential oil and methanol extract of *Achillea wilhelmsii* C. Koch (Asteraceae) against *Anopheles stephensi* Liston (Diptera: Culicidae), a malaria vector

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Abstract

Background: Mosquitoes are responsible for the transmission of many pathogens and parasites and consequently serious diseases in humans. Currently, application of plant derivatives has been suggested as an alternative bio-control technique for these medically important vectors.

Methods: In this study the essential oil and methanol extract of *Achillea wilhelmsii* were tested against late-3rd or young-4th instar larvae stages of mosquito vector, *Anopheles stephensi*, under laboratory condition. The larval mortality was calculated after 24 h of the exposure period. Data were subjected to Probit analysis in order to estimate the lethal concentration for 50% and 90% of mortality values.

Results: Results showed that the essential oil induced 100% larval mortality of *An. stephensi* larvae after 24 h with a dosage of 160 ppm, However, a dosage of 320 ppm of methanol extract was required to reach 100% larval mortality.The essential oil methanol extract exerted significant larvicidal activity with LC_{50} values of 39.04 and 115.73 ppm, respectively.

Conclusion: Our finding suggests that *A. wilhelmsii* oil is a potential source and has valuable larvicidal compounds for mosquito larval control.

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Introduction

Mosquitoes (Culicidae Diptera) play an important role in the transmission of several human diseases such as malaria, yellow fever, dengue fever and filariasis (1).

Malaria is one of the most important parasitic diseases transmited to humans by Anopheles mosquitoes. Malaria is still a major cause of death and severe illness in many parts of the world. According to World Health Organization report (2015), there have been approximately 214 million cases of malaria worldwide and 438,000 deaths due to malaria, mostly in children and in Africa (2).

Malaria is one of the most important vector-borne diseases in Iran (3). Recent studies on anopheline mosquitoes in Iran have reported the presence of 31 Anopheles species, eight of them have important roles in human malaria (4).

Among these species, *Anopheles (Cellia) stephensi* Liston 1901 is considered as a primary vector of malaria in the South and Southeast of Iran, especially in Sistan and Baluchistan, Hormozgan and southern parts of Kerman Province. *An. stephensi* is an important vector of human malaria throughout the Middle East and South Asia, including the Indo-Pakistan subcontinent (5,6).

Vector control strategy is one of the most important strategies in malaria elimination program that has been started in Iran and focuses on application of larvicides, indoor residual spraying and long-lasting insecticidal nets (7,8).

Currently, Mosquito larval control using larvicidal agents is a major part of controlling mosquitoes-borne diseases. The most commonly used larvicides are organophosphorus compounds such as temephos, fenthion, and chlorpyrifos (1). However, their toxicity to aquatic organisms as well as the environment, the insecticide resistance of arthropods and severe acute and chronic poisoning are being increasingly reported (9-12); therefore, it seems necessary to find out new larvicidal compounds from alternative sources such as plants.

Recently, botanical insecticides have been applied in vector control due to their efficacy, degradability and safety. Numerous plant products have been reported as insecticides for mosquito control (13).

Many plant oils showed a broad spectrum of activity against pest insects ranging from insecticidal, antifeedant, repellent, oviposition deterrent and growth regulatory activities (14).

Recent studies have demonstrated the insecticidal properties of a variety of essential oils from several plant genera such as *Bunaum*, *Cionura*, *Stachys*, *Lawsonia*, *Eucalyptus*, *Thymus*, *Kelussi*a, *Heracleum*, *Foeniculum*, *Coriandrum*, *Cupressus*, *Tagetes*, *Citrus* and *Zhumeria* against larvae of *An. stephensi* (15-24).

Achillea wilhelmsii C. Koch is a wild aromatic herb belonged to Asteraceae family with average

size of 15–40 cm. This plant is widely found in different parts of Iran (25).

The main components of *A. wilhelmsii* essential oil are 1,8-cineole, camphor, borneol and linalool (26,27). In recent studies, the essential oil obtained from *A.wilhelmsii* has been evaluated for antifungal (27,28) antioxidant (26), antimicrobial (29), insecticidal (30) and repellent (31) activities.

In the current study, we examined the larvicidal effect of essential oil and methanol extract obtained from *A. wilhelmsii* against *An. stephensi* larval stage under laboratory conditions.

Collection of plant material

Fresh leaves and flowers of *A. wilhelmsii* were collected in July 2015 from central Zagros mountain in the Chahar Mahal & Bakhtiari region, Iran (50° 46° E, 32° 19° N, elevation:2099 m above the sea level). The plant was identified and authenticated by a plant taxonomist. Voucher specimen was deposited at the Department of Biology, Shahrekord University.

Preparation of the methanol extract (MeOH)

The collected leaves and flowers of the plant were shade dried at room temperature (23–25° C) and ground in a manual mill. The powder was passed through a sieve. About 100 gr of powder was extracted with aqueous methanol MeOH. H_2O at ratio 80:20 (v/v) using percolation method for 48 h. The extract was filtered through a Buchner funnel with No.1 Whatman filter paper. The solvents were removed using a rotary evaporator at 60ºC under vacuum. The extract was dried using vacuum oven at 60ºC and was kept in dry clean black glass bottle at +4ºC for further use.

Isolation of the essential oil (EO)

The air-dried leaves and flowers of plants were submitted for 3 h to water-distillation using a Clevenger-type apparatus. The obtained EO was dried over anhydrous sodium sulphate and after filtration, stored at +4º C until tested.

Mosquitoes

The malarial vector, *A. stephensi* (Bandar-Abass strain), was reared in the laboratory of Department of Medical Entomology and vector Control, Hormozgan University of Medical Sciences. The larvae were fed with powdered fish food. Mosquitoes were held at 28±2 °C, 65±5 % relative humidity, with a light period of 12-h light/12-h dark.

Acute toxicity against mosquito larvae

The larvae between third and fourth stages were used for experiments. The larval bioassays were carried out according to the larval susceptibility test method suggested by WHO (32).

The plant essential oil and methanol extract and first were dissolved in ethanol and methanol, respectively and then serial dilution (10, 20, 40, 80,160 and 320 ppm) of them were prepared. Twenty-five larvae (in a 250-ml beaker) of early

fourth instar stage were used for larvicidal assay, and four replicates were maintained for each concentration. During this experiment, no food was offered to the larvae. In the control beakers only solvent was dissolved in water. The larval mortality was calculated after 24 h of the exposure period. Data were subjected to Probit analysis in order to estimate the lethal concentration for 50% and 90% mortality (LC_{50} and LC_{90}) values (33).

Results

The yield of *A. wilhelmsii* EO was 0.3 (v/w based on dry weight). The EO was yellow with a distinct sharp odor. The results of larvicidal activity of EO and methanol extract of *A. wilhelmsii* against late-3rd or young-4th instar larvae of *A. stephensi* were presented in Table 1.

Results showed that the EO induced 100% larval mortality of *A. stephensi* larvae after 24 h with a dosage of 160 ppm; however, a dosage of 320 ppm of methanol extract was required to reach 100% larval mortality (Fig 1).

Table 1. The mortality rate of *Anopheles stephensi* larvae at different concentrations of *A. wilhelmsii* essential oil and methanol extract

Dosage (ppm)	Essential Oil				Methanol Extract			
	Subjects	**Responses**	**S.D**	**Mortality (%)**	**Subjects**	**Responses**	**S.D**	**Mortality (%)**
Control	100	0	0	0	100	1	0.5	1
10	102	3	0.96	2.94	100	0	0	0
20	105	17	1.5	16.19	102	2	0.58	1.96
40	107	58	2.94	54.21	100	9	1.26	9
80	103	82	2.65	79.61	101	34	2.65	33.66
160	100	100	0	100	104	53	3.3	50.96
320	102	102	0	100	100	100	0	100

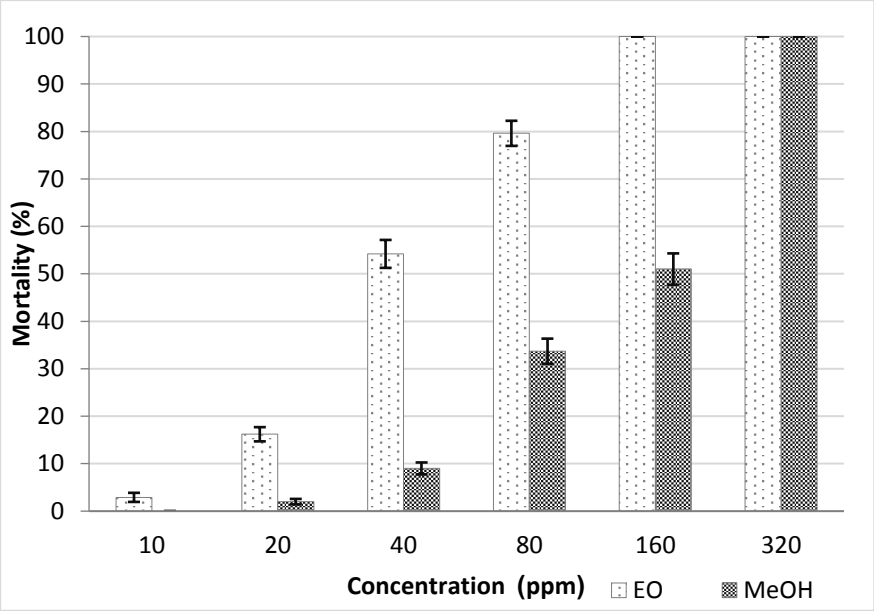

Figure 1. Percentage of larval mortality of A. *stephensi* after treatment with essential oil and methanol extract of *A. wilhelmsii*.

Both essential oil and methanol extract exerted significant larvicidal potential against *An. stephensi* after exposure for 24 h .The LC_{50} values of 39.04 and 115.73 ppm and LC_{90} values of 95.4 and 302.3 ppm were obtained for EO and methanol extract respectively. The probit regression parameters of *An. stephensi* exposed to different interval concentrations of EO and methanol extract are shown in Table 2.

In regression line, a positive correlation was observed between the concentration of dose (X) and the probit mortality (Y) (Fig. 2).

Table 2. Probit parameters of *Anopheles* stephensi to essential oil and methanol extract of A. *wilhelmsii* at different interval concentrations.

Products	A	B ± SE	LC_{50} 95%C.I.	LC_{90} 95 %C.I.	X^2 (df)	p value
Essential oil	-5.26	3.3 ± 0.235	39.04	95.4	4.8 (3)	>0.05
Methanol extract	-6.34	3.07 ± 0.63	115.73	302.3	22.8 (3)	<0.05

A: y-intercept; B: the slope of the line; SE: standard error; LC_{50}, 95 % C.I; lethal concentration causing 50 % mortality and its 95 % confidence interval; LC_{90}, 95 % C.I: lethal concentration causing 90 % mortality and its 95 % confidence interval; X^2: heterogeneity about the regression line; df: degree of freedom; p value, represent heterogeneity in the population of tested.

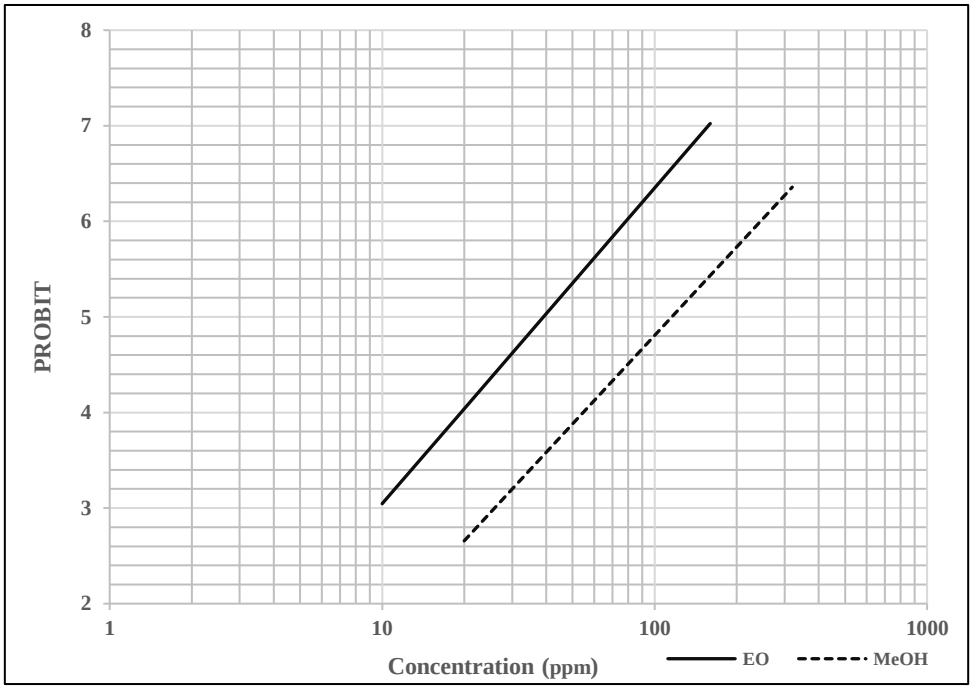

Figure 2. Probit regression line of *Anopheles stephensi* to essential oil and methanol extract of A. *wilhelmsii* at different interval concentrations.

Discussion

Botanicals may be alternative sources to synthetic insecticides for mosquito control, because they constitute a rich source of bioactive compounds that is potentially suitable for pest control. Therefore, the screening of local medicinal plants in order to be used as mosquito larvicide may lead to the production of effective larvicides in mosquito control programs. The yield of oil obtained from *A. wilhelmsii* (0.3%w/v) is similar to what has been reported in a recent study (34). The results obtained from this study showed that the essential oil and methanol extract of *A. wilhelmsii* have larvicidal effects, however essential oil was more effective (3-folds) than methanol extract against larvae of *An. stephensi*.

In a similar study, Sedaghat et al. investigated larvicide activity of essential oil and methanol extract of *Eucalyptus camaldulensis* against *A. stephensi*. They found that the essential oil was about 4.5-folds more effective than methanol extract (23). The larvicide activity of essential oil and methanol extract of *Cionuraerecta* were investigated by Mozaffari et al. and they found that essential oil (LC_{50}: 77.30) had a significant effect against larvae *An. stephensi* (3.2-folds) compared with methanol extract (LC_{50}: 250.38) (16).

According to the obtained results, *A. wilhelmsii* oil was very effective against *An. stephensi* with LC_{50} value of 39 ppm. According to the proposed classification for larvicidal activity of plant oils

against mosquito larvae, *A. wilhelmsii* has been considered as an active plant (18).

The results of the current study are comparable with what have been reported for other essential oils in the literature and shows that EO of *A. wilhelmsii* exhibited stronger larvicidal activity against *An. stephensi* larvae.

In a study by Tiwary et al. on the larvicidal activity of EO of *Zanthoxylumarmatum* against *An. stephensi*, the LC_{50} was calculated as 58 ppm (35). Similarly, Raj et al. have reported that the LC_{50} value of 53.9 for EO of *A. wilhelmsii* against *An. stephensi* larvae (36). Sedaghat et al. reported LC_{50} values of respectively 104.80 and 120.95 ppm for EO of *Heracleumpersicum* and *Coriandrumsativum* against *An. stephensi* (17). Lastly, EO extracted from *Cupressusarizonica* was tested against *An. stephensi* larvae and LC_{50} value of 79.30 ppm was reported (22). In conclusion, the present study showed that the essential oil of *A. wilhelmsii* may have a potential role in the control of larvae of *An. stephensi*. Further studies with isolation of bioactive constituents might lead to safer and more effective products in mosquito control.

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