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Original article

Larvicidal activity of *Ferulago carduchorum* Boiss. & Hausskn. against the main malaria vector, *Anopheles stephensi*

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Abstract

Background and objectives: Malaria is a remarkable cause of death in the world. Several ways are used by researchers to control or to decrease the detrimental effects of the disease, in which natural insecticides with less malaria vectors resistance and lower environmental damages are considered in last decades. The objective of this study was to assess the larvicidal activity of the whole flowering samples of *Ferulago carduchorum* Boiss. & Hausskn against the main malaria vector *Anopheles stephensi*. **Methods**: The partition fractions of ethyl acetate, chloroform, methanol, and the total 80% methanol extract from *Ferulago carduchorum* were examined for larvicidal activity against late 3rd and early 4th instar larvae of malaria vector *Anopheles stephensi.* The LC_{50} and LC_{90} values were calculated by probit analysis. **Results**: The LC_{50} of the total extract, chloroform, ethyl acetate and methanol fractions were 0.4799, 0.2361, 0.7437 and 3.7017 ppm, respectively. The LC_{90} of the total extract, chloroform, ethyl acetate and methanol fractions were 1.5090, 0.4547, 1.8918 and 10.8857 ppm, respectively. **Conclusion**: On the basis of the presence of nonpolar compounds in the chloroform fraction, we can propose that the larvicidal activity of this fraction would be due to these compounds. The extract might be useful for improvement of new natural insecticides.

Keywords: *Anopheles stephensi*, *Ferulago carduchorum* Boiss. & Hausskn., larvicidal activity

Introduction

Malaria is one of the most important problems in developing countries [1]. Every year 300-500 million cases are affected by malaria among which, 2-3 million of whom (mainly children and pregnant women) lose their life. According to WHO reports which was published in 2013, about 219 million cases of malaria and 660000 deaths were observed in 2010, despite the effort made to control the disease. About 17000 cases a year are observed in Iran mainly in provinces of Sistan and Baluchestan, Hormozgan and in some parts of Kerman [2]. There are eight anopheline vectors in this area including *A. culicifacies, A. stephensi*, *A. dthali*, *A. fluviatilis, A. superpictus*,

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A. pulcherrimus [3,4], *A. sacharovi* and *A. maculipennis*, which the last two are considered as malaria vectors in northern part of the country [5,6]. *A. stephensi* is considered as the main malaria vector in southern of Iran [7]. There are several measures for malaria vector control in Iran including using larvicids, indoor residual spraying and use of treated bed nets. Resistance to insecticides is widespread for mosquitoes and many other pests, causing operational problems for control programmers. Thus, researchers are trying to find new insecticides based on natural constituents throughout the world [8]. *Ferulago* is a genus from Apiaceae family; there are 35 species of this genus in the world, some of which are found in Iran, including *F. stellata, F. angulata, F. macrocarpa, F. subvelutina, F. bernardi, F. phialocarpa, F. contracta* and *F. carduchorum*. Some species of *Ferulago* have been used ethnically as sedative, tonic and remedy of chronic ulcers, snakebites, hemorrhoids, diseases of the spleen, headaches and digestive pains [9]. *Ferulago carduchorum* Boiss. & Hausskn (Apiaceae) grows in west part of the country [10]. It is used by local people in dairy and oil ghee to increase hold time and give a pleasant taste. In the past, the species was used as a natural preservative to delay expiration date of meat. Various biological activities have been reported for the genus *Ferulago*, so far including antioxidant activity for *F. angulata* [11], larvicidal activity for *F. nodosa* [12], cytotoxicity for *F. angulata* [13], antibacterial and anti-*Candida* properties for *F. angulata* subsp. *carduchorum* [14]. The present study was aimed to determine the larvicidal activity of the extract of *F. carduchorum* against the main malaria vector *A. stephensi*.

Experimental

Plant material

The aerial parts of *F. carduchorum* were collected from Manesht Mountain, Ilam provices of Iran in the flowering stage. The plant was identified by Dr. Y. Ajani and voucher specimen was deposited at the Central Herbarium of the Institute of Medicinal Plants, Karaj, Iran.

Extraction and fractionation

The plant aerial parts were dried for a week at room temperature and powdered. The air-dried and ground aerial parts of *F. carduchorum* were extracted using the percolation method with MeOH/H_2O (80/20) at room temperature for 3 weeks. The extract was evaporated with a rotary evaporator and freeze-dried. Then, it was subjected to silica gel (mesh 230-400) column chromatography (CC) with *n*-hexane, $CHCl_3$, EtoAc and MeOH as an eluents, to give four different fractions.

Mosquitoes

Anopheles stephensi larvae were obtained from the laboratory of the School of Public Health and Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran (originally from the malarious areas of Kazeroon, Fars province Iran). They were reared under insectary conditions at 60±5% humidity, 25±1 ºC and 12:12 hours dark: light photo-period and were fed with 10 percent sucrose solution. The late 3rd and early 4th instar larvae were used for the tests.

Biological study

Extract of *F. carduchorum* was assessed against late 3rd and early 4th instar larvae of *A. stephensi*. These larvae were exposed to different concentrations of the *F. carduchorum* extracts which were prepared in methanol. The minimum concentration was 20 ppm (chloroform fraction) and the maximum was 2560 ppm (methanol fraction). These concentrations gained the appropriate mortality to plot the regression line. Mortality rate was calculated after 24 h exposure period. All the tests were conducted at 25±1 ºC and 60±5% humidity, and 12:12 hours (dark: light) photo- periods in the laboratory conditions. For each concentration, 4 replicates of 25 larvae were used. The *n*-hexane fraction could not be dissolved in common solvents (methanol and

ethanol) of larvicidal tests, so the related biological test was not carried out in the study.

Statistical analysis

LC_{50} and LC_{90} were determined by the use of regression line applied by Finny [15]. From the regression line between probit mortality and logarithmic dose, the parameters including LC_{50}, confidence interval (CI), LC_{90} and slope values were calculated [16]. The regression line was plotted using Microsoft Excel

Results and Discussion

In the present study, the larvicidal activity of the total extract and chloroform, ethyl acetate and methanol fractions were investigated.

As it is mentioned in figure 1, both LC_{50} and LC_{90} values for chloroform fraction were 0.2361 and 0.4547 ppm, respectively which were lower than other extracts. The total extract of *F. carduchorum* showed good activity with LC_{50} and LC_{90} values 0.4799 and 1.5090 ppm, respectively. LC_{50} and LC_{90} values for the ethyl acetate and methanol fractions were (0.7437, 1.8918 ppm), and (3.7017, 10.8857 ppm), respectively. The larvicidal activity of the total extract and chloroform fraction could be attributed to their coumarins and phytosteroids. Secondary metabolites with broad range of activities have been found in genus of *Ferulago*.

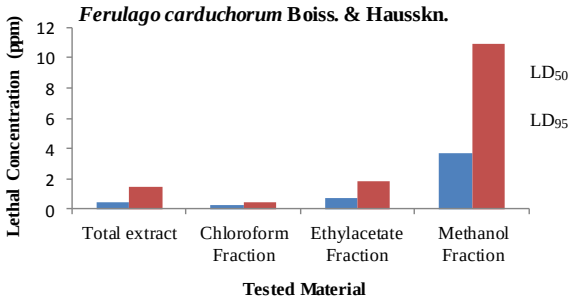

Figure 1. Lethal concentration values of extract and fractions from *Ferulago carduchorum* Boiss. & Hausskn. against third instar larvae of *Anopheles stephensi*

In our other researches, the larvicidal activities of methanol extracts of some Iranian plants including *Eucalyptus camaldulensis* [17], *Lawsonia inermis*, *Thymus kotschyanus*, *Cedrus deodara*, eight species from *Stachys* [18] and *Nepeta menthoides* [19] against *A. stephensi* were investigated. Comparing to the results of other studies mentioned above, it seems that the total extract and fractions of *F. carduchorum* were more potent than these plants against *A. stephensi*. Therefore, *F. carduchorum* could be suggested as a considerable alternative insecticide in comparing with chemical compounds. The previous studies confirmed the presence of phytosteroids, coumarins and flavonoids in *Ferulago* genus [20]. So, the larvicidal activity of *F. angulate* may be due to the high content of coumarins and phytosteroids. The chloroform fraction of *F. carduchorum* was found to be more effective than other fractions and the total extract**.** In conclusion, the larvicidal effects of the chloroform fraction could be related to the semi- to nonpolar compounds existing in the plant.

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Declaration of interest

The author declares that there is no conflict of interest. The author alone is responsible for the content of the paper.

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