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# *Astrodaucus persicus* as a new source of bioinsectisides against malaria vector, *Anopheles stephensi*

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ABSTRACT

**Objective:** To determine the larvicidal activities of petroleum ether, chloroform, ethyl acetate and methanol fractions of roots and fruits extracts of *Astrodaucus persicus* from Apiaceae family against malaria vector, *Anopheles stephensi* (*An. stephensi*).
**Methods:** Twenty five third instar larvae of *An. stephensi* were exposed to various concentrations (10–160 g/L) of fractions and were assayed according to World Health Organization protocol. The larval mortality was calculated after 24 h treatment.
**Results:** Among tested fractions, the highest larvicidal efficacy was observed from ethyl acetate fraction of fruits extract with 50% and 90% mortality values ($LC_{50}$ and $LC_{90}$) of 34.49 g/L and 108.61 g/L, respectively. Chloroform fraction of fruits extract was the second larvicidal sample with $LC_{50}$ of 45.11 g/L and $LC_{90}$ of 139.36 g/L. Petroleum ether fractions of fruits and roots and methanol fraction of fruits showed moderate toxicity against *An. stephensi*.
**Conclusions:** *Astrodaucus persicus* is a potential source of valuable and natural larvicidal compounds against malaria vector, *An. stephensi* and can be used in mosquitoes control programs as an alternative to synthetic insecticides.

## 1. Introduction

Malaria, the most important vector-borne disease, is still endemic in the south of Iran and more than 100 countries and kills 1.5–2.7 million people every year according to World Health Organization (WHO) reports [1–3]. Mosquitoes are the main factors of vector-borne diseases including malaria therefore transmission of diseases can be interrupted by various chemical control methods [4]. *Anopheles stephensi* (*An. stephensi*) Lison 1901, as main vectors of human malaria, is one of the six anopheline mosquitoes in southern parts of Iran [5,6]. Use of larvicidals, indoor residual spraying and treated bed nets are some of several solutions for malaria vector control in Iran [7]. Because of the resistance of mosquitoes, possible toxicity hazards, environmental pollution and adverse effects on the non-target flora and fauna which were created by extensive consumption of chemical insecticides, the researchers are screening for finding new sources of insecticides [8,9]. Insecticidal natural products especially from plant origin have been used for control of different pest insects and vectors in recent years because of their innate biodegradability and less harmful effects on non-target organisms [10].

*Astrodaucus persicus* (*A. persicus*) (Boiss.) Drude (Apiaceae) is one of the native species of this genus in different parts of Iran and neighbouring countries [11]. The young roots or aerial parts of *Astrodaucus* were used as food additives or salad vegetables [12]. There were many reports about chemical composition of *A. persicus* essential oils. Some of them showed blue colour and potent antioxidant activities [13]. The only biological study on *A. persicus* exhibited roots and aerial parts of this plant have potent antiproliferative effects against breast cancer [14,15].

The present investigation was based on larvicidal assessments of petroleum ether, chloroform, ethyl acetate and methanol fractions of fruits and roots extracts of *An. persicus* against third instar larvae of malaria vector, *An. stephensi* Liston.

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## 2. Materials and methods

### 2.1. Plant materials

Different parts of *A. persicus* including ripe fruits and roots were collected from Zanjan province in September 2015. A sample of plant was identified by Dr. Y. Ajani and deposited in Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences (6553-TEH).

### 2.2. Extraction and fractionation

A total of 150 g of fruits and roots of *A. persicus* were powdered separately and macerated with 80% methanol to conclude 17.0 g fruits extract and 5.5 g roots extract. Fruits and roots extracts were fractionated with different solvents to obtain fruits and roots petroleum ether (2.9 g and 2.0 g), chloroform (0.8 g and 1.0 g), ethyl acetate (0.3 g and 0.3 g) fractions and the residue named fruits and roots methanol fraction (11.5 g and 2.0 g), respectively.

### 2.3. Mosquito culture

The *An. stephensi* larvae of Bandar Abbas strain were obtained from the insectarium of Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences. They were maintained at controlled conditions of 29–30 °C temperature, 70%–80% relative humidity under a 12/12 h light/dark photoperiod cycle. The third instar larvae were selected for the experiment. The adults were fed *ad libitum* with 10% sucrose solution 14 h prior to the tests when it was withdrawn from the cage.

### 2.4. Larvicidal bioassay

The susceptibility of mosquito larvae to various fractions of fruits and roots of *A. persicus* was measured according to WHO standard protocols [16] with slight modification in our laboratory. All fractions were tested at 160, 80, 40, 20 and 10 g/L dilutions in a final volume of 250 mL of water in a 500 mL capacity of glass beaker. A total of 25 larvae were exposed to each concentration of samples and were controlled (containing methanol and tween as emulsifier).

The numbers of dead larvae were counted after 24 h of continuous exposure, and both dead and moribund larvae were considered as dead; the mortality percentage was reported from the average of four replicates.

### 2.5. Statistical analysis

The corrected mortality percentages of the treated larvae were analysed using Abbott's formula [17]. The data were analysed by log-probit method according to Finney [18] and all the parameters including lethal concentration to cause 50% and 90% mortality values ($LC_{50}$ and $LC_{90}$, respectively), 95% confidence limits (CL) and regression equations were calculated. Statistical differences between means were considered significant with $P < 0.01$.

## 3. Results

Table 1 showed the results of the bioassay tests of *A. persicus* fruits and roots fractions on the *An. stephensi* larvae in various interval concentrations. Chloroform and ethyl acetate fractions of fruits extract at concentration of 160 g/L induced the best larvicidal effects with the rate of 98.0% and 97.0%, respectively. The mortality of mentioned fractions was 65.4% and 82.6% at

**Table 1**
Larvicidal effect of different concentrations of *A. persicus* fruits and roots fractions against third instar larvae of *An. stephensi*.

| Extract | Fractions | Mortality percentage (%) | | | | |
|---|---|---|---|---|---|---|
| | | 10 (g/L) | 20 (g/L) | 40 (g/L) | 80 (g/L) | 160 (g/L) |
| Fruits | Petroleum ether | 8.5 | 19.0 | 24.2 | 43.7 | 74.0 |
| | Chloroform | 8.1 | 11.4 | 49.0 | 65.4 | 98.0 |
| | Ethyl acetate | 11.5 | 22.9 | 55.4 | 82.6 | 97.0 |
| | Methanol | 7.0 | 12.1 | 29.3 | 47.1 | 58.3 |
| Roots | Petroleum ether | 5.5 | 12.4 | 17.2 | 24.8 | 80.4 |
| | Chloroform | 2.9 | 10.2 | 23.8 | 39.6 | 54.3 |
| | Ethyl acetate | 1.0 | 1.9 | 1.0 | 19.0 | 20.6 |
| | Methanol | 2.9 | 9.8 | 24.7 | 39.3 | 50.5 |
| Control | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

**Table 2**
Probit regression line parameters of the response of *An. stephensi* larvae to different fractions of *A. persicus* fruits and roots extracts.

| Extract | Fractions | $LC_{50}$ (g/L) | 95% CL (LCL–UCL) | $LC_{90}$ (g/L) | 95% CL (LCL–UCL) | Regression equation | $\chi^2$ (df) |
|---|---|---|---|---|---|---|---|
| Fruits | Petroleum ether | 82.53 | 68.65–103.03 | 513.60 | 342.05–920.65 | Y = 1.61X–3.09 | 5.958* (3) |
| | Chloroform | 45.11 | 27.51–77.39 | 139.36 | 80.28–615.22 | Y = 2.62X–4.33 | 16.03* (3) |
| | Ethyl acetate | 34.49 | 30.63–38.77 | 108.61 | 91.06–135.60 | Y = 2.57X–3.95 | 2.850* (3) |
| | Methanol | 102.98 | 82.96–136.33 | 759.62 | 460.90–1606.90 | Y = 1.48X–2.97 | 1.631* (3) |
| Roots | Petroleum ether | 101.21 | 85.10–125.25 | 478.94 | 33.63–799.90 | Y = 1.90X–3.81 | 29.100* (3) |
| | Chloroform | 125.06 | 100.51–167.17 | 798.39 | 491.51–1579.28 | Y = 1.59X–3.34 | 1.034* (3) |
| | Ethyl acetate | 476.44 | 286.27–1251.84 | 3335.62 | 1265.18–22599.18 | Y = 1.52X–4.06 | 9.250* (3) |
| | Methanol | 134.82 | 105.87–188.03 | 945.20 | 549.33–2176.24 | Y = 1.51X–3.23 | 2.212* (3) |

$LC_{50}$: Lethal concentration to cause 50% mortality in population; $LC_{90}$: Lethal concentration to cause 90% mortality in population; CL: Confidence limit; LCL: Lower confidence limit; UCL: Upper confidence limit; $\chi^2$ (df) = *Chi* square (degrees of freedom); *: Significant at $P < 0.01$.

80 g/L and 49.0% and 55.4% at 40 g/L, respectively. It is obvious that ethyl acetate fraction of fruits have better larvicidal activity in lower concentrations. The petroleum ether fractions of roots and fruits exhibited mortality induction with rate of 80.4% and 74.0% at 160 g/L.

The parameters of probit regression lines of different fruits and roots fractions of *A. persicus* for 24 h exposure against *An. stephensi* including $LC_{50}$ and $LC_{90}$ values, 95% confidence interval and their regression equations were presented in Table 2. The highest sensitivity of third instar larvae to ethyl acetate fraction of fruits extract was also evident by their lowest LC values ($LC_{50}$ of 34.49 g/L and $LC_{90}$ of 108.61 g/L). Chloroform fraction of fruits extract was the second larvicidal sample with $LC_{50}$ and $LC_{90}$ of 45.11 g/L and 139.36 g/L, respectively. Petroleum ether fractions of fruits and roots and methanol fraction of fruits extracts showed moderate toxicity against *An. stephensi* with $LC_{50}$ value of 82.53 g/L, 101.21 g/L and 102.98 g/L, respectively.

## 4. Discussion

Screening and isolation of natural compounds possessing pesticidal activity led to find phytochemicals acting as general toxicants (insecticide/larvicides) against both adult and immature larval stages of mosquitoes. Some of these secondary metabolites interfere with growth and evolution (growth inhibitors) or reproduction (chemosterilant) of insects, or acting as repellent or attractant by stimulation of olfactory [19]. These categories of pesticides with natural origin are known as biopesticides and are also eco-friendly when they are used as an alternative method for reduction of environmental side effects of chemical pesticides [8,20,21].

It was evident from our results that different fractions of fruits extract of *A. persicus* had better insecticide potentials than roots extract. Ethyl acetate, chloroform and petroleum ether fractions of fruits presented their potent larvicidal effects while only one fraction (petroleum ether) of roots showed such activity. The behaviour of all fractions of *A. persicus* except ethyl acetate fraction of roots extract were dose-dependent against mosquito larvae meaning that a rise in the concentration of fractions was the principle cause of mortality in *An. stephensi* larvae. According to the regression equations it was obvious that chloroform and ethyl acetate fractions of fruits extract of *A. persicus* had greater slopes than other means and they reached to higher mortality percentage in lower concentrations; as it was seen ethyl acetate and chloroform fractions of fruits had lower $LC_{50}$.

In recent years, a variety of plant extracts and essential oils and their secondary metabolites have been reported to exhibit insecticidal activities against *An. stephensi* [22–24]. Some bioactive components with 1, 3-benzodioxole structure have been isolated from hexane fraction of roots extract of *A. persicus* [25]. The molecules containing benzodioxole ring exhibited anti-parasitic [26], antimalarial [27] and antioxidant effects [28]. They are also used as herbicides, pesticides or pesticide intermediates [29]. It seems that these structures affect both malaria parasites and vectors, therefore, *A. persicus* can be considered as a new source for malaria control. In addition, the genera of plants deposited in Apiaceae family are known as rich sources of coumarin compounds. There were many reports about larvicidal activity of plants from Apiaceae family and coumarin compounds [30,31]. Fruits and roots of *A. persicus* have compounds with coumarin structures, and with its insecticidal activities these components can also make contributions.

In conclusion, this study revealed that some fruits and roots fractions of *A. persicus* have potentials of mosquitoes control, so they could be selected for further investigations about identification of effective components and their mechanisms of actions and eventually development of a new bio-rational insecticide.

## Conflict of interest statement

The authors declared that they have no conflict of interest.

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