# Larvicidal Activities of Some Iranian Native Plants against the Main Malaria Vector, *Anopheles stephensi*

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**Abstract-** Malaria is considered a major health problem in Iran. There are different methods for vector control. In this study we tested the larvicidal effects of some Iranian plants. The methanolic extracts of 11 plants were prepared with percolation method. The larvicidal activities of them against malaria vector, *Anopheles stephensi* were studied using World Health Organization standard method. All $LC_{50}$ values of methanolic extracts of plants that we screened were lower than 300 ppm. The methanolic extract of aerial parts of *Lawsonia inermis* and *Stachys byzantina* showed high larvicidal activity with $LC_{50}$ values 69.40 ppm and 103.28 ppm respectively. The results obtained from this study suggest that the methanolic extracts of these plants have larvicidal effects against *Anopheles stephensi* larvae and could be useful in the search for new natural larvicidal compounds.



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**Keywords**: *Anopheles stephensi;* Iranian plant; Larvicidal activities; Methanolic extract

## Introduction

Mosquitoes transmit many serious human diseases such as malaria, filariasis, yellow fever, dengue and other viral diseases. There are about 3500 species of mosquitoes, grouped into three subfamilies (1). *Anopheles stephensi* is urban and rural mosquito in Iran (2). Malaria is considered as one of the most important health problem in Iran especially in southern parts. In the south parts of Iran there are six anopheline vectors including *Anopheles culicifacies*, *An. stephensi*, *An. dthali*, *An. fluviatilis*, *An. superpictus* and *An. pulcherrimus* (3-11). *Anopheles sacharovi* and *An. maculipennis* can transmit human malaria in northern part of the country (12-15) Dichloro-diphenyl-trichloroethane (DDT), dieldrin, pyrethrum, lindane, heptachlor as larvicides have been used in the past (16). Chemicals larvicides could be carcinogenic, mutagenic and teratogenic for humans. The nonstop use of chemical larvicides has often led to the disorder of the natural biological control system (17). There are some reports about the resistance to these chemicals in mosquitoes. Therefore we need to identify alternative insecticide substances from natural products. Many scientists reported insecticidal activities of plants belong to different families in different parts of the world. There are several native reports about crude solvent extracts of different parts of plants, essential oils or their chromatographic fractions. They showed various levels of bioactivity against different developmental stages of malaria vectors (18). Some plants have phytochemicals constituents for the control of mosquitoes. One of the earliest reports of the use of plant extracts against mosquito larvae is extraction of plants' alkaloids like nicotine, anabasine, methyl anabasine and lupinine from the Russian weed in 1933(19). Some plant families such as *Asteraceae*, *Cladophoraceae*, *Labiatae*, *Meliaceae*, *Oocystaceae* and *Rutaceae* have the maximum potential for development of novel mosquito control agents (20).

The genus *Lawsonia* has one species, *Lawsonia inermis* (21,22). Henna`s leaves, flowers, seeds, stem barks and roots had been used in Iran to treat some

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diseases such as rheumatoid arthritis, headache, ulcers, diarrhea, leprosy, fever, leucorrhoea, diabetes, cardiac disease. It had hepatoprotective effect and been used as colouring agent too (23-25).

The genus *Thymus* has 200 species which are medicinal plants and are distributed through Mediterranean regions (26). Fourteen species of *Thymus* are introduced as Iranian flora, among which, four are native to Iran. Different species of *Thymus* have different types of components with different percentages. Mainly they contain thymol and carvacrol as main essential oil constituents, phenolic compounds such as rosmarinic acid which may have anti-edemic and macrophage-inhibiting effects, as well as flavonoids (27-30).

Using anti-inflammatory and anti-microbial drugs for different causes with different side effects allow human to use herbal drugs with equal effects (28). *Thymus* has different therapeutic effects such as anti-dyspepsia, antibacterial, anti-hypertension (31), antispasmodic and sedative (32), diuretic, treatment of pediatric enuresis and anti-acne (33). It has been experimented for anti-bacterial and anti-fungal activity, anti-depressant (27,29,31,34), anti-arthralgia as well as anti-tussive effects (35).

The genus *Stachys* has more than 270 species and is one of the most important plants of the *Labiatae* (36). The genus *Stachys* includes 34 species in Iran (37).

Phytochemical investigations of *Stachys* species have shown the occurrence of flavonoids, diterpenes, phenylethanoid glycosides and saponins (38). This genus has some effect to treat genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers (39). Many studies have shown various activities in this genus such as anti-inflammatory (36,38,40,41), anti anxiety (42), antibacterial (43,44), anti-nephritic (45), anticancer (46,47) and anti-*Helicobacter pylori* (48) and antioxidant effects (49-51). Some species of this genus are used in folk medicine, specially *Stachys paalustri*s L. and *S. sylvatica* L. which are approved for healing wounds, treating abdominal pains and as disinfectant, anti-spasmodic and anti-fever (51).

*Cedrus deodara* (family: Pinaceae) is a great evergreen tree. Its bark has some effect such as anti-inflammation, anti-arthritic pain and anti dermal diseases (53). It also used as anti-spasmodic and anti-cancer against human epidermoid carcinoma of nasopharynx (54), anti-fungal, anti-allergic, analgesic, anti-oxidant, anti-filarial and molluscicide (55). The objective of this study was to elucidate the larvicidal activity of *Lawsonia inermis, Thymus kotschyanus, Cedrus deodara* and eight species from *Stachys*, including: *S. trinervis, S. inflata, S. setifera, S. laxa, S. persica, S. subaphylla, S. byzanthina* and *S. turcamanica.*

## Materials and Methods

### Collection, preparation and processing of leaf extracts of test plants

Leaves of *Lawsonia inermis, Thymus kotschyanus, Cedrus deodara* and eight species from *Stachys*, including: *S. trinervis, S. inflata, S. setifera, S. laxa, S. persica, S. subaphylla, S. byzanthina* and *S. turcamanica* were collected from different area of Iran in June 2009 (Table 1). Other representative samples of the plant including flower and fruits were also collected and species identification was carried out at the Herbarium of the Department of Pharmacognosy, faculty of Pharmacy, Tehran University of Medical Sciences. Voucher specimens were deposited in the same Herbarium.

### Processing of the plant material

Arial parts of plants were air dried under shade and were then powdered. The powder material was macerated with methanol 80% in 1:10 (w/v) in percolator at room temperature for 3×48 hours. The extracts of plants were filtered through cotton and subsequently with Whatman filter paper (12.5 cm size). Rotary evaporator was used to remove methanol 80% from the extract. The crude extracts were collected in small vials and stored in -4ºC deep freeze until used in mosquito larvicidal tests.

### Mosquito larvae

*Anopheles stephensi* which is susceptible to all larvicides were obtained from the insectary of School of Public Health, Tehran University of Medical Sciences. Larvicidal tests were performed based on WHO standard method (WHO, 2011) (56). Mosquitoes were reared using standard procedures. The mosquito colony was maintained continuously at 27°C with 12:12 light and dark photoperiod at 65% ± 5% relative humidity. Late third and early fourth instar larvae were used for all the tests.

### Larvicidal bioassays

Larvicidal bioassays were conducted for 24 hours in glass beakers of 400 ml test solutions with 4 replicates of each test concentration 40, 80, 160, 320 and 640 ppm for methanolic extracts of plants. Batches of 25 late third

instar larvae of *An. stephensi* were transferred into each test concentration of crude methanol leaf extract by means of droppers. Larval mortalities were recorded after 24 hours of exposure in each separated concentration of extract.

Dead and moribund larvae were counted at each concentration and then were polled. Controls included batches of larvae exposed to 1 ml of solvent alone.

**Data analysis**

$LC_{50}$ (lethal concentration to cause 50% mortality in the population) and $LC_{90}$ (lethal concentration to cause 90% mortality in the population) were determined by plotting the regression line as described by Finney (57). The percentage mortality was calculated using Abbot's formula (58).

## Results

Methanolic extracts from Iranian plants were studied for natural insecticides effect instead of synthetic agents. The larvicidal activities of the extracts against mosquito larvae under laboratory conditions are given in tables 1. All plants extract exhibited significant larvicidal activity against *An. stephensi* (Table 1).

In this study 11 extract of plants were tested, and their $LC_{50}$ and $LC_{90}$ values are reported in table 1. All extracts which were screened had $LC_{50}$ values smaller than 300 ppm (Table 1). Moreover, probit regression lines for 11 extract are shown in figure 1. Methanol extracts of *L. inermis* and *S. byzantina* showed the strongest toxicity against *An. stephensi*.

**Table1.** Parameters of probit regression line *Anopheles stephensi* to methanol extract derived from different plants.

| NO | Specimens | Place of Collecting | A | B ± SE | $LC_{50}$ , 95% C.I. | $LC_{90}$, 95% C.I. | $X^2$ (df) | *P*-value |
|---|---|---|---|---|---|---|---|---|
| 1 | *Thymus vulgaris* | Tehran | -6.95 | 3.05±0.46 | 129.08<br>191.33<br>288.01 | 324.42<br>503.98<br>1405.14 | 13.25 (3) | <0.05 |
| 2 | *Lawsonia inermis* | Kerman | -6.57 | 3.57±1.62 | *<br>69.40<br>* | *<br>158.75<br>* | 52.39 (2) | <0.05 |
| 3 | *Cedrus deodara* | Ahvaz | -7.52 | 3.57±0.49 | 81.89<br>128.04<br>206.9 | 187.56<br>292.87<br>1149.73 | 6.03 (2) | <0.05 |
| 4 | *Stachys trinervis* | Halejerd, Karaj | -6.50 | 2.8±0.39 | 145.15<br>210.42<br>317.36 | 381.25<br>604.04<br>1693.24 | 11.01 (3) | <0.05 |
| 5 | *Stachys inflata* | Ardebil | -9.72 | 4.24 ±1.81 | *<br>195.84<br>* | *<br>392.81<br>* | 43.73 (2) | <0.05 |
| 6 | *Stachys setifera* | Ardebil | -10.06 | 4.45±1.17 | *<br>181.62<br>* | *<br>352.35<br>* | 17.55(2) | <0.05 |
| 7 | *Stachys laxa* | Golestan Forest | -8.92 | 3.67±0.3 | 244.32<br>269.64<br>298.16 | 519.93<br>602.6<br>729.53 | 2.32 (2) | >0.05 |
| 8 | *Stachys persica* | Ardebil | -12.03 | 1.14±1.12 | 20.63<br>282.80<br>7620.36 | 310.20<br>515.94<br>* | 14.89(2) | <0.05 |
| 9 | *Stachys subaphylla* | Golestan Forest | -8.32 | 3.46 ±0.54 | 144.49<br>252.60<br>459.1 | 360.02<br>592.37<br>4051.32 | 7.47(2) | <0.05 |
| 10 | *Stachys byzantina* | Ardebil | -6.02 | 2.99±0.53 | 62.63<br>103.29<br>182.34 | 163.04<br>276.99<br>1292.33 | 17.9(3) | <0.05 |
| 11 | *Stachys turcamanica* | Golestan Forest | -9.18 | 3.82 ±0.31 | 230.21<br>253.45<br>279.37 | 477.08<br>549.05<br>657.87 | 2.09 (2) | >0.05 |

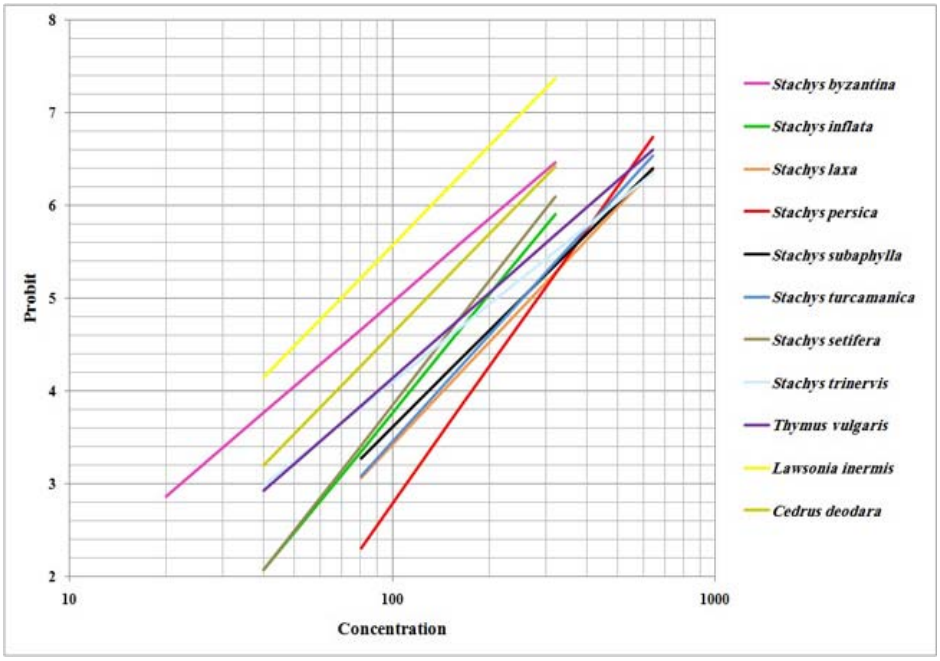


**Figure 1.** Probit regression line of *An. stephensi* exposed to different interval concentrations derived from different plants.

## Discussion

Most botanical components are rapid acting and breakdown quickly in the environment. The extract of whole leaf and essential oil of some certain plants have been investigated against some public health pests (59-63).

The use of botanical pesticide may help in reducing the environmental side effects by the synthetic insecticides. The results obtained suggest that the extracts of 11 plants may be a promising as larvicide against *An. stephensi*. There are many researches in the field of phytochemical investigation of *L. inermis*. It has naphthoquinone derivatives, phenolic compounds, terpenoids, sterols, aliphatic derivatives, xanthones, coumarin, fatty acids, amino acids and other constituents. Naphthoquinone fraction obtained from leaves of *L. inermis* showed significant immunomodulatory effect. Quinonic compounds from henna were studied *in vitro* for anti-microbial properties. Lawsone isolated from the leaves of *L. inermis* has shown significant anti-fungal antibiotic effect (64). Phytochemical investigation on *Stachys* species has shown the occurrence of flavonoids, diterpenes, phenyl ethanoid glycosides and saponin (38). In *S. byzantina* some compounds were identified such as tritriacontane, hentriacontane, oleic acid, stigmasterol and lawsaritol. *S. byzantina* showed anti-bacterial activities and flavonoids are responsible of these effects (65) and also show anti-inflammatory effects (38).

Larvicidal effect of different extracts from the plants was studied on *An. stephensi* larvae. In 2005 Hajiakhundi *et al*. reported that the $LC_{50}$ and $LC_{90}$ values of the methanolic extract from *Tagetes minuta* L. against *An. stephensi* larvae were 2.5 mg/l and 11 mg/l respectively (62).

In the same study larvicidal activity of *Eucalyptus camaldulensis* against *An. stephensi* was performed. Results showed that the $LC_{50}$ and $LC_{90}$values were 89.85 mg/l and 397.75 mg/l respectively (66). In another study Sedaghat *et al.* reported that essential oil of *Cupressus arizonica*, was one of the toxic plants on *An. stephensi* larvae (67). Also its $LC_{50}$ and $LC_{90}$ were 79.30 mg/l and 238.89 mg/l, respectively (67). Sedaghat *et al.* studied oils from *Heracleum persicum*, *Foeniculum vulgare* and *Coriandrum sativum* at much lower concentrations and reported $LC_{50}$ values equivalent to 104.8, 20.1 and 120.95 mg/l, respectively (68).

In other investigation, Nathan *et al*. (69) reported that the larvicidal activity of essential oil from *Eucalyptus tereticornis* Sm. with $LC_{50}$ and $LC_{90}$ values were 23.8 and 63.9 ppm respectively against *An. stephensi* larvae.

Therefore, the present study revealed that the methanol extracts of *L. inermis* and *S. byzantina* exhibited high larvicidal activities as 69.40 ppm and 103.28 ppm respectively. These results could be useful in the search for novel, more selective, and

biodegradable compounds for the control of the malaria vector *An. stephensi*.

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