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Larvicidal activity of essential oil and methanol extract of *Nepeta menthoides* against malaria vector *Anopheles stephensi*

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ABSTRACT

Objective: To investigate the larvicidal activity of essential oil and methanol extract of the *Nepeta menthoides (N. menthoides)* against main malaria vector, *Anopheles stephensi* (*An. stephensi*). **Methods:** The essential oil of plant was obtained by Clevenger type apparatus and the methanol extract was supplied with Percolation method. Larvicidal activity was tested by WHO method. Twenty five fourth-instar larvae of *An. stephensi* were used in the larvicidal assay and four replicates were tested for each concentration. Five different concentrations of the oil and extract were tested for calculation of LC_{50} and LC_{90} values. **Results:** The LC_{50} and LC_{90} values were determined by probit analysis. LC_{50} was 69.5 and 234.3 ppm and LC_{90} was 175.5 and 419.9 ppm for the extract and essential oil respectively. **Conclusions:** According to the results of this study methanolic extract of plant exhibited more larvicidal activity than essential oil. This could be useful for investigation of new natural larvicidal compounds.

1. Introduction

Malaria is an important cause of death and illness in children and adults, especially in tropical countries [1]. According to the latest report of World Health Organization, it kills between 1.5-2.7 million people every year [2]. It remains as a main public health problem in southern part of Iran which involved three provinces of Sistan and Baluchistan, Hormozgan and tropical areas of Kerman province [3]. The annual malaria cases have been arrived from 66075 to 6211 during 1995-2009 indicating good decline of disease [4]. There are six anopheline vectors in this area including *Anopheles culicifacies (An. culicifacies)*, *Anopheles stephensi* (*An. stephensi*), *Anopheles dthali (An. dthali)*, *Anopheles fluviatili (An. fluviatili)*, *Anopheles superpictus (An. superpictus)* and *Anopheles pulcherrimus (An. pulcherrimus)* [5,6]. *Anopheles sahacrovi* (*An. sahacrovi*) and *An. maculipennis* can transmit human malaria in northern part of the country [7,8].

An. stephensi Liston 1901 is known to be an important urban malaria vector in the middle-east and Indian subcontinent. This species considered as main malaria vector in southern of Iran [9]. There are several measures for malaria vector control in Iran including larviciding, indoor residual spraying and use of treated bed nets [3]. Resistance to such insecticides is widespread in mosquitoes and many other pests, causing operational problems for control programmers [10]. Several botanicals offer great promise as sources of phytochemicals for the control of mosquitoes. Six plant families with several representative species, Asteraceae, Cladophoraceae, Labiatae (Lamiaceae), Meliaceae, Oocystaceae and Rutaceae, appear to have the greatest potential for providing future mosquito control agents [11]. The extract of whole leaf and essential oil of certain plants have been investigated, and showed toxic effect against some public health pests [12-15].

Nepeta (Lamiaceae) with about 250 species, widely spread in different geographical region such as Asia, North America, North Africa, temperate Europe and in the Mediterranean region. There are 67 species of *Nepeta* in

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Iran in which 39 species are native [16,17]. *Nepeta* species have anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activity [18], and can be used as antispasmodic, diuretic, febrifuge, diaphoretic and for tooth trouble, kidney and liver disease [16]. The other effects of *Nepeta* species are analgesic, anticancer, antialzheimeran, antiseptic, antispasmodic, antitussive, carminative, digestive, laxative and sedative [19]. This genus is also studied for larvicidal effect [20-26]. Many of these properties are related to terpenoids and flavonoides that exist in this genus [27]. Nepetalactones, 1,8-cineole, α-pinene, α-terpineol and caryophyllene oxide, were the main compounds that have been found in the oil of almost all the studied species of genus *Nepeta* [28].

Nepeta menthoides (N. menthoides) , the case of this study, is one of native species that grows in north-west of Iran (Azarbaijan, Tabriz and Sabalan mountain) [29]. In Iran, this plant is named as Ostokhodus in folk medicine and is used for gastrodynia, sedation, high blood pressure, bone pain nervous disorders, rheumatism and blood depurative [27,30]. In order to survey the effects of *N. menthoides*, in this study we investigated the larvicidal activity of the plant for the first time in the world.

2. Materials and methods

2.1. Plant materials

The aerial parts of *N. menthoides* were collected from Gurgur rainfall, Sareein road, Ardabil province of Iran in the flowering stage in July 2010. The plant was identified by Dr. Y. Ajani and voucher specimen has been deposited at the Central Herbarium of the Institute of Medicinal Plants (ACECR), Karaj, Iran. (Herbarium Number: 1447).

2.2. Essential oil isolation

A total of 1000 g of under shade dried and powdered aerial parts of *N. menthoides* were subjected to hydrodistillation using a modified Clevenger-type apparatus for 4 h. The oil was dried over anhydrous sodium sulphate and transferred into amber-colored vials at 5 ℃ for further work.

2.3. Preparation of methanolic extract

A total of 150 g of dried and powdered aerial parts of *N. menthoides* was extracted by methanol 80% (3×800 mL) at room temperature for two weeks. After removal of the solvent in vacuum at 50 ℃ by Rotary evaporator, the residue (22 g, 14.7% w/w) was stored at 4 ℃ in sealed vials until usage.

2.4. Mosquito rearing

The fourth-instar larvae of *An. stephensi* Bandar-Abass strain was obtained from the Department of Medical Entomology, Tehran University of Medical Sciences. The mosquito colony was maintained continuously at 27 ℃ with 12:12 light and dark photoperiod in (80±10)% relative humidity. Larvae of *An. stephensi* were continuously available for the mosquito larvicidal experiments.

2.5. Bioassays and larval mortality

Bioassays were performed according to the standard method recommended by the World Health Organization (WHO) [31]. The fourth-instar larvae of *An. stephensi* were exposed to essential oil at different interval concentrations of 80, 120, 180, 270 and 405 ppm and methanol extract at 12.5, 25, 50, 100 and 200 ppm for 24h. In the control beakers only 1 ml of solvent (Ethanol for essential oil and methanol for extract) was dissolved into the water. Mortality was counted after 24 hours recovery period.

2.6. Analysis method

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 the use of regression line employed by Finney [32]. The percentage mortality was calculated by using the formula and corrections for mortality when necessity were done by using Abbot's formula [33].

3. Results

The hydrodistillation of aerial parts of *N. menthoides* gave yellowish oil in 0.3% (w/w) yield, based on the dry weight of the plant. After examination of different concentration, essential oil in concentration of 405 ppm, and methanol extract in 200 ppm showed 100% mortality. By testing other concentration and drawing the regression line, LC_{50} and LC_{90} were calculated. LC_{50} were 69.5 and 234.3 ppm and LC_{90} were 175.5 and 419.9 ppm for extract and essential oil respectively.

Table 1 shows the parameters of probit regression line of *An. stephensi* larval susceptibility to methanol extract and essential oil of *N. menthoides* at different concentration. Moreover, probit regression lines for two essential oil and extract are shown in Figure1.

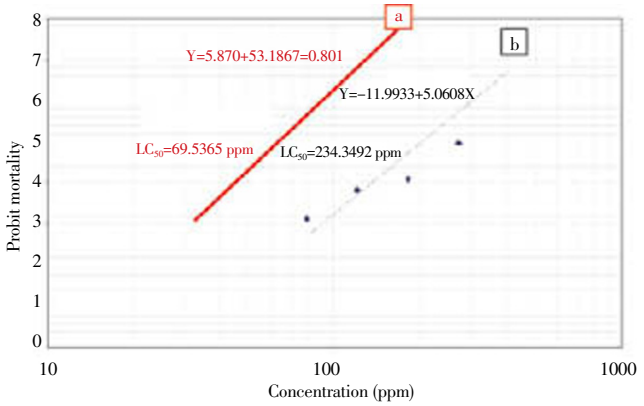

Figure 1. Regression line of extract of *N. menthoides* against *An. stephensi*.

a= Methanol extract

b= Essential oil

Table 1

Probit regression line parameters of essential oil and extract of N.menthoides against *An. stephensi*

specimens	a	b±SE	LC_{50} (ppm) , 95% C.I.	LC_{90} (ppm) , 95% C.I.	χ^2 table (df)	*P*-Value
Methanol extract	-5.8705	3.1867±0.8010	69.5365(19.1285-148.2744)	175.5456(96.5155-5022.3493)	16.266 (3)	0.001
Essential oil	-11.9933	5.0608±1.3080	234.3492(139.0987-507.9856)	419.8614(279.4273-9309.4599)	16.266 (3)	0.001

a = intercept, b±SE = slope±standard error, LC50±95%C.I.= lethal dose cause 50% mortality, 95% confidence interval, Lc90±95%C.I.= lethal dose cause 90% mortality, 95% confidence interval, (df) = degree of freedom, p= p value

Table 2

Lavicidal activity of some plants against *An. stephensi*.

Plant Name	Part of use	LC_{50} ppm	LC_{90} ppm	Reference
Tagetes minuta	Essential oil of Fresh plant	1.0532	3.83	42
	Essential oil of Dried plant	1.3015	5.07	
Tagetes minuta	Total Extract of aerial part	2.50	10.97	43
Melia azedarach	Total Extract of aerial part	5.50	34.90	23
Calotropis procera	Fresh latex	13.06	23.53	22
Centaurea bruguierana	Petroleum ether fraction	15.70	48.30	44
Foeniculum vulgare	Essential oil of seeds	20.10	44.51	45
Cupressus arizonica	Total Extract of aerial part	79.30	238.89	46
Heracleum persicum	Essential oil of seeds	104.80	174.22	45
Calotropis procera	Total Extract of aerial part	109.71	234.61	22
Coriandrum sativum	Essential oil of seeds	120.95	389.90	45
Cymbopogon olivieri	Essential oil of aerial part	321.90	983.60	24

4. Discussion

The use of plant essential oils and extract in vector control is a suitable alternative method for reduction of the side effects of chemical pesticides on the environment [34]. In some previous studies the larvicidal activity of plant extracts and essential oils were investigated against *An. stephensi*.

According to the results of present study, larvicidal activity of the extract and essential oil of *N. menthoides* showed that the methanol extract with LC_{50}=69.5 ppm had must significant larvicidal effect in comparison to essential oil (LC_{50}=234.3 ppm), Where as the essential oil of Tagetes minuta, Heracleum persicum, Foeniculum vulgare and Coriandrum sativum, had presented lower LC_{50} than *N. menthoides* essential oil and Cymbopogon olivieri, were known with higher LC_{50} and weaker larvicidal effects.

Also the extract of Tagetes minuta, Melia azedarach and petroleum ether fraction of Centaurea bruguierana, showed lower LC_{50} and more toxicity, but Calotropis procera and Cupressus arizonica extracts had higher LC_{50} and lower toxicity than our extract.

The essential oil of the sample was analyzed in parallel study by GC/MS. Twenty one compounds representing 92.88% of the total oil were identified, in which 4a α ,7 β ,7a α -Nepetalactone (18.39%), 4a α ,7 α ,7a α -Nepetalactone (17.57%) and 1,8 cineol (16.66%) were reported as the main compounds [35]. In another report about the oil of this plant also Nepetalactone isomers (36.85%) and 1,8cineol (31.29%) were the major compounds [36]. There is a report about the isomers of Nepetalactone that have shown feline attractant and mosquitoes repellency which is 10 times more powerful than DEET (N, N-diethyl-m-toluamide) [37,38]. In another study on biological activity of Nepeta parnassica oils and isolated Nepetalactones, both oil and isolated Nepetalacton had significant toxicity on *Pogonomyrmex* sp. ants. Also in feeding bioassay it was shown that Nepetalacton had more toxicity than the oil [28]. In a research that was done by Mills, 1,8 cineol as another major compound of Nepeta species, showed a significant acetylcholinesterase inhibitor effect that attributed to insecticidal activity [39].

So we expected that Nepetalacton isomers and 1,8 cineol as major compounds of N. menthoides extract and the oil must be mentioned as effective compounds related to larvicidal activity. Also it seems that the amount of Nepetalacton and 1,8cineol in the extract are higher than the oil.

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