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## Wild chive oil is an extremely effective larvicide against malaria mosquito vector *Anopheles stephensi*

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### ABSTRACT

**Objective:** To assess the chemical composition and mosquito larvicidal potentials of essential oil of wild chive (*Allium schoenoprasum* L.) against *Anopheles stephensi*.

**Methods:** In the search for an environmentally safer alternative mosquitoes control, the larvicidal efficacy of essential oil obtained from the leaves of *Allium schoenoprasum* L. against *Anopheles stephensi* was determined. The composition of chive essential oil was analyzed by gas chromatography–mass spectrometry.

**Results:** In toxicity assays, the essential oil demonstrated substantial larvicidal activity against larvae of *Anopheles stephensi* with LC<sub>50</sub> and LC<sub>90</sub> values of 2.60, and 7.31 ppm after 24 h of exposure, respectively. Gas chromatography–mass spectrometry analysis of chive essential oil identified 35 components representing more than 97.31% of the total essential oil. The main constituents were sulfur compounds, including diallyl trisulfide (13.72%), 2-ethyl[1,3]dithiane (8.93%), allyl methyl trisulfide (8.77%), and trimethylene trisulfide (6.64%), respectively.

**Conclusions:** This study demonstrated that wild chive essential oil has a rich source of eco-friendly bioactive compounds for use as a mosquito larvicide. The main reason for its extraordinary properties may be related to the high percentage of sulfur compounds.

## 1. Introduction

Mosquitoes are dangerous vectors of numerous serious human pathogens, causing millions of deaths every year[1]. Some species within the genera *Anopheles*, *Aedes*, and *Culex* are responsible for the most deadly and debilitating mosquito-borne diseases[2].

Malaria is one of the major diseases that transmitted by *Anopheles* mosquitoes[1]. According to the latest World Health Organization (WHO) estimates, at least 216 million new cases of malaria in 2016 occurred worldwide resulting in more than 445 000 deaths[3]. The *Anopheles (Cellia) stephensi* Liston 1901 is one of the most important

malaria vectors[2,4].

A more efficient strategy to reduce mosquito populations is to target the larvae. Currently, the control of mosquito larvae relies upon on using synthetic insecticides[2]. Continued use of such insecticides for mosquitoes control programs has brought on disruption of natural biological systems and has sometimes caused the development of resistance in mosquitoes[5,6].

Based on this view, new compounds must be found in vector

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control that will replace synthetic insecticides. Secondary metabolites of plants are candidates for the discovery of new products to fight mosquitoes[7,8]. Botanical pesticides appear to have no adverse effect on non-target organisms. Insecticide-based on plants has the advantage of presenting novel modes of action against mosquito vectors that could lessen the risk of cross-resistance[7]. In this context, essential oils (EOs) have received much attention as a potential source of bioactive compounds against mosquitoes[9].

Plants belonging to *Allium* genus possess a very long tradition of ethnobiological uses, as foods, spices, medicinal herbs, and food preservatives. *Allium schoenoprasum* L. (*A. schoenoprasum*) (chive), is one of the members of this genus. The species of the genus *Allium* are characterized by the presence of their rich content in sulfur compounds[10]. The chive leaves contain some sulphur compounds[11]. In recent studies, the EO obtained from *A. schoenoprasum* has been evaluated for antimicrobial[12], antioxidant[12,13], antifungal[14], anthelmintic[15] and anticancer[16] activities.

In this study, we examined the toxicity of *A. schoenoprasum* EO against *Anopheles stephensi* (*An. stephensi*). In addition, the constituents of EO were identified and quantitatively determined by gas chromatography–mass spectrometry (GC-MS) analyses.

## 2. Materials and methods

### 2.1. Plant material

Fresh leaves of *A. schoenoprasum* L. were collected before flowering stage from different areas of naturally growing areas in May 2017 from Marghmalek region, Chaharmahal and Bakhtiari Province, Iran (50° 25' E, 32° 25' N elevation: 2 490 m). The plant was identified

and sample voucher was deposited at the Department of Biology, Shahrekord University (SKU), Shahrekord, Iran. The leaves were dried in a dry and shady place and kept in a light protected container.

### 2.2. Essential oil extraction

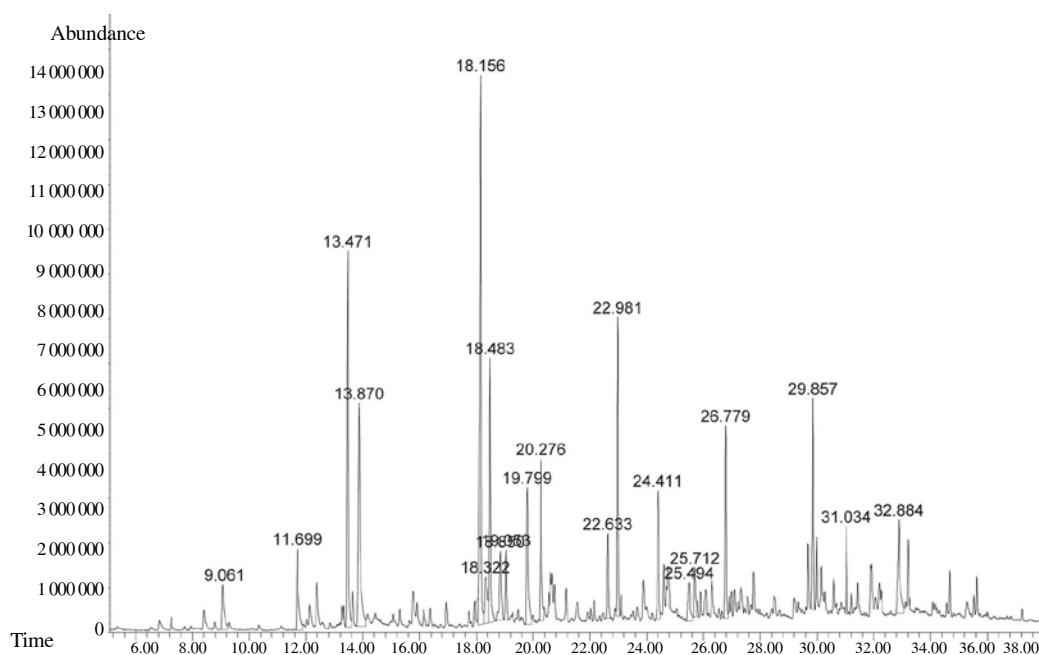
The dried leaves (400 g) were extracted by hydro-distillation for 3 h, using a Clevenger-type. The yields were measured according to dry weight. The obtained EO was dried over sodium sulfate anhydrous and kept in a refrigerator at 4 °C till required.

### 2.3. Essential oil analysis

The EO obtained from *A. schoenoprasum* was analyzed using an Agilent 6890/5973 N GC-MS equipped with an Agilent 19091S-433 HP-5 MS capillary column. The carrier gas was helium (1.2 mL/min). In split mode, a 1 µL volume of each sample has been injected and the split ratio was 1: 20. The GC oven program was as follows: initial temperature of 50 °C for 3 min, programmed rate 3 °C/min up to 300 °C. The temperatures of the injector and detector were 250 °C and 280 °C, respectively. An ionizing energy of 70 eV has been used for MS detection. The Kovat's indices (KI) for the detected compounds were determined in relation to a homologous series of *n*-alkanes (C8- C24) running under the same operating conditions. Tentative identification of the compounds was based on computer matching with the Wiley and NIST libraries, as well as by comparing the mass spectrum and retention indices (RI) with those reported within the literature[12,17,18].

### 2.4. Mosquito rearing

The mosquito, *An. stephensi* was reared in the Culicidae insectary,



**Figure 1.** Total ion chromatogram of GC-MS analysis of the essential oil from leaves of *Allium schoenoprasum*.

Hormozgan University Medical Sciences. The mosquito colonies were kept up at  $(28 \pm 1)^\circ\text{C}$  with 12:12 light and dark photoperiod in  $(70 \pm 5)\%$  relative humidity. The larval stage of *An. stephensi* were continuously accessible for the bioassay tests.

### 2.5. Toxicity of essential oil against mosquito larvae

The larvicidal bioassays of the *A. schoenoprasum* EO were carried out according to procedures prescribed by the World Health Organization[19]. In brief, twenty-five larvae (in a 250 mL beaker) at the late 3rd and early 4th instars of *An. stephensi* were exposed to 0.625, 1.25, 2.5, 5 and 10 ppm of EO in water for 24 h. Four replicates were performed for each concentration (100 larvae). Larval mortality at 24 h after exposure was recorded.

### 2.6. Data analysis

The  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values, regression equation and the 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL) and *Chi*-square values were determined by using probit analysis (Finney's method)[20, 21].

## 3. Results

### 3.1. Yield and chemical components of the essential oil

The yield of EO from *A. schoenoprasum* was 0.005% (v/w) based on dry weight. The chemical components and their relative percentage of *A. schoenoprasum* EO were shown in Table 1.

GC-MS identified 35 constituents representing about 97.31% of the EO (Figure 1). The main constituents were sulfur compounds, including diallyl trisulfide (13.72%), 2-ethyl[1,3]dithiane (8.93%), allyl methyl trisulfide (8.77%) and trimethylene trisulfide (6.64%), respectively. Other minor components were found to be  $\beta$ -ionone (5.97%),  $\beta$ -eudesmol (4.53%), and allyl methyl tetrasulfide (4.22%).

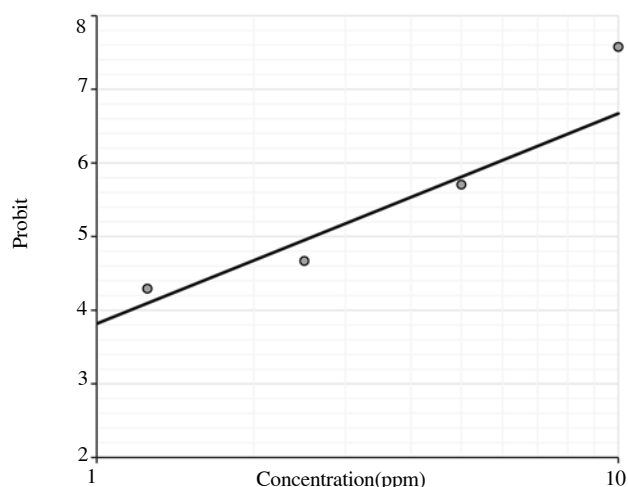
### 3.2. Mosquito larvicide effect

The EO of wild chive exerted significant larvicidal activity against *An. stephensi* after exposure for 24 h. A positive correlation between EO concentration and mosquito mortality has been observed (Figure 2). EO induced  $(5.00 \pm 0.96)\%$ ,  $(24.00 \pm 0.82)\%$ ,  $(37.00 \pm 1.89)\%$ ,  $(76.00 \pm 2.16)\%$  and 100% larval fatality at 0.625, 1.25, 2.5, 5 and 10 ppm, respectively. The obtained values for  $\text{LC}_{50}$  and  $\text{LC}_{90}$  were 2.6 (Lower: 1.69-Upper: 4.01) and 7.31 (Lower: 4.57-Upper: 21.89) ppm, respectively; the Probit equation was  $Y = -1.1832 + 2.8532X$ . The *Chi*-square value is significant at  $P < 0.05$  level ( $\chi^2$ : 13.08, *df*: 3). The *Chi*-square value in the bioassay tests indicated probably the heterogeneity of the test population.

**Table 1.** Chemical constituents of the essential oil from leaves of *Allium schoenoprasum*

Peak No.	Components <sup>a</sup>	RI <sup>b</sup>	Composition (%)
1	3,4-Dimethylthiophene	890	0.25
2	Dimethyl trisulfide	980	0.52
3	1,3-Dithiane	995	1.92
4	cyclohexanone, 2,2,6 trimethyl-	1 035	0.15
5	Diallyl disulphide	1 078	2.80
6	Allyl propyl disulfide	1 085	0.47
7	$\beta$ -Linalool	1 100	1.45
8	2-Ethyl[1,3]dithiane	1 132	8.93
9	Allyl methyl trisulfide	1 138	8.77
10	<i>L</i> -camphor	1 141	0.92
11	Trimethylene trisulfide	1 145	6.64
12	Dimethyl tetrasulphide	1 214	0.13
13	$\beta$ -Cyclocitral	1 225	1.44
14	4,6-Dimethyl-[1,2,3] trithiane	1 239	0.17
15	Isobornyl acetate	1 284	0.11
16	Diallyl trisulfide	1 295	13.72
17	Carvacrol	1 302	2.40
18	Dipropyl trisulfide	1 330	7.25
19	3,5-Diethyl-1,2,4-trithiolane	1 338	3.66
20	Allyl methyl tetrasulfide	1 393	4.22
21	Geranyl acetone	1 462	0.26
22	$\beta$ -Farnesene	1 469	1.92
23	$\beta$ -Ionone	1 495	5.97
24	5-methyl-2-phenylhex-2-enal	1 525	0.57
25	Diallyl tetrasulphide	1 558	3.50
26	1-(1-Propenylthio)propyl propyl disulfide	1 583	1.59
27	4,6-Diethyl-1,2,3,5-tetrathiolane	1 594	1.48
28	Geranyl isovalerate	1 606	1.12
29	$\beta$ -eudesmol	1 658	4.53
30	Ar-turmerone	1 665	1.36
31	Hexahydrofarnesyl acetone	1 856	1.43
32	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	1 923	0.31
33	Hydrofol	1 974	3.94
34	Palmitic acid	1 999	1.88
35	Methyl linolenate	2 119	1.53
Total			97.31

<sup>a</sup>Compounds listed in order of elution from a HP-5MS column; <sup>b</sup>RI: Retention indices as determined on HP-5MS using the homologous series of *n*-alkanes.



**Figure 2.** Probit regression line of *Anopheles stephensi* exposed to different interval concentrations of essential oils of *Allium schoenoprasum*.

#### 4. Discussion

The *Allium* genus produces sulfur-based chemicals which are extremely potent in terms of aroma and physiological effects[22, 23]. Forever previous investigations have also revealed that the sulfur compounds in EOs of *Allium* spp. could inhibit acetylcholinesterase enzyme activity in insects[24, 25]. Our results show that more than 66% of the compounds of the chive oil are comprised of sulfur volatiles. diallyl trisulfide (13.72%), 2-ethyl[1,3]dithiane (8.93%), and allyl methyl trisulfide (8.77%) which are known as the main components of the EO of wild chive. The presence of these mentioned compounds in chive oil may be responsible for the larvicide effect on *An. stephensi*.

Previous research conducted by Mnayer *et al.*[12] showed that the main constituents in the EO of bulbs of chive collected from France were dipropyl disulfide, dipropyl trisulfide, and methyl propyl trisulfide which including about 19.49%, 15.21%, and 8.47% of total EO, respectively. In another report, the EO of the leaves of chive from Venezuela mainly contained bis-(2-hydroxyethyl)-disulfide[72.06], 2,4,5-trithiahexane (5.45%), and tris-(methylthio)-methane (4.01%)[18]. It is known that several factors such as genetic and environmental conditions, seasonal variation, plant age, phenological stages and isolation methods influence the composition of EO produced by same-species plants[26].

According to the bioassay tests, chive EO has exerted significant larvicidal potent against *An. stephensi*, so that 10 ppm of it was effective enough to cause 100 % larval mortality.

Chive EO possessed very strong larvicidal activity (LC<sub>50</sub>: 2.6 ppm) against *An. stephensi* compared with the other EOs using the same bioassay method in previous investigations, *e.g.* EOs of *Zhumeria majdae* (LC<sub>50</sub>: 61.34 ppm)[27], *Citrus paradise* (LC<sub>50</sub>: 35.71 ppm) [28], *Bunium persicum* (LC<sub>50</sub>: 27.72 ppm)[29], *Tanacetum persicum* (LC<sub>50</sub>: 48.64 ppm)[30], *Achillea kellalensis* (LC<sub>50</sub>: 35.42 ppm)[30], *Satureja bachtiarica* (LC<sub>50</sub>: 24.27 ppm)[31], *Achillea wilhelmsii* (LC<sub>50</sub>: 39.04 ppm)[32], *Cionura erecta* (LC<sub>50</sub>: 77.30 ppm)[33], *Zanthoxylum armatum* (LC<sub>50</sub>: 58 ppm)[34], *Nigella sativa* (LC<sub>50</sub>: 53.9 ppm)[35], *Clausena anisate* (LC<sub>50</sub>: 119.59 ppm)[36]. Prajapati *et al.* reported the mosquito larvicidal efficacy of EOs from 10 medicinal herbs with LC<sub>50</sub> values ranging from 110.2 ppm to 387.8 ppm against *An. stephensi*[37]. In this paper, which is based on previous studies about the use of plants EOs to fight against mosquito larvae, there are eight ratings for mosquito larvicidal activity of plant EOs. And EO of *A. schoenoprasum* lies in the second group extremely active. If we consider Sedaghat *et al.* suggestion in the regard, this oil was thought to be a highly active larvicide[38].

It seems that the mosquito larvicidal properties of chive oil are related to their content of volatile sulfur compounds. Organosulfur compounds, such as diallyl sulfide and diallyl disulfide, have demonstrated insecticidal effects on pest insects[39, 40]. Kimbaris *et al.* studied the biological activity of several garlic oil ingredients; their results showed diallyl disulfide showed good larvicidal activity against *Culex pipiens* with LC<sub>50</sub> of 6.09 ppm, while the garlic EO had an LC<sub>50</sub> value of 8.01 ppm[41]. Plant EOs have favorable

ecotoxicological properties, making them suitable to be used as pesticide in vector control programs in the future[7].

This study demonstrated that the EO obtained from wild chive has a rich source of eco-friendly bioactive compounds for use as a mosquito larvicide. The main reason for its extraordinary properties might be related to the high percentage of sulfur compounds, including diallyl trisulfide, 2-ethyl[1,3]dithiane, allyl methyl trisulfide, and trimethylene trisulfide, which can be used as a natural larvicidal agent for breeding-site management in the mosquito control programs.

In that way, the findings of the current investigation provide a possible way for further studies to find out the active molecule. However, further investigations must be conducted to describe the mode of action of each constituent separately and also its effects on non-target organisms.

#### Conflict of interest statement

The authors declare that no conflict of interest exists.

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