

Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima* Mozaffarian essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae)

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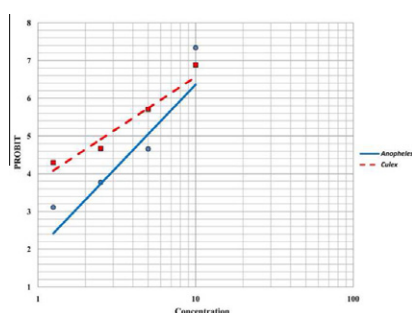
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HIGHLIGHTS

- ▶ The larvicidal activity of essential oil of *Kelussia odoratissima* was evaluated.
- ▶ The main constituents of the oil were Z-ligustilide (77.73%).
- ▶ The result suggest that *K. odoratissima* oil has potential source of larvicidal compounds.
- ▶ A guideline provided with six categories for larvicidal activity of plant essential oils.
- ▶ This plant could be considered as a highly active plant.

GRAPHICAL ABSTRACT



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ABSTRACT

The larvicidal activity of essential oil extracted from an indigenous plant, *Kelussia odoratissima* Mozaffarian was evaluated against two mosquito species, *Anopheles stephensi* and *Culex pipiens*. The chemical composition of the essential oil obtained by hydrodistillation from branch tips and leaf of this plant was determined by GC and GC/MS analysis. Forty-nine constituents were identified in the oil. The main constituents of the oil were Z-ligustilide (77.73%), 2-octen-1-ol acetate (6.27%), E-ligustilide (2.27%) and butylidene phthalide (1.97%).

Five different logarithmic concentrations of essential oil were evaluated against the 4th instar larvae of *An. stephensi* and *Cx. pipiens*. The LC_{50} and LC_{90} values against *An. stephensi* larvae were 4.88 and 9.60 ppm and for *Cx. pipiens* were 2.69 and 7.90 ppm, respectively.

These properties suggest that *K. odoratissima* oil has potential source of valuable larvicidal compounds for mosquito larval control. This plant which causes high mortality at lower dose could be considered as a highly active plant. In this paper a guideline suggested for larvicidal activity of plant essential oils.

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1. Introduction

Mosquitoes are considered as one of the most important group of arthropods from the medical and veterinary entomology point of view. They act as vectors of several diseases, like malaria, yellow

fever, dengue, filariasis, encephalitis and make some serious health problems to humans (Youdeowei and Service, 1983).

Human malaria caused by an infection with protozoa of the genus *Plasmodium* continues to be the most important vector-borne disease which is transmitted only by females of the genus *Anopheles*. It is estimated that malaria is responsible for 781 000 deaths and 225 million infections globally in 2009 (WHO, 2010). Currently, more than 25 recognized *Anopheles* species found in Iran while seven of them have important roles in malaria

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transmission (Sedaghat et al., 2003a,b; Vatandoost et al., 2004; Sedaghat and Harbach, 2005). Among these species, *Anopheles (Celia) stephensi* Liston 1901 is considered as a primary vector of malaria in the southern parts of the country (Vatandoost et al., 2004, 2006; Sedaghat and Harbach, 2005; Oshaghi et al., 2006; Hanafi-Bojd et al., 2011; Mehravaran et al., 2012). Similarly, *Culex (Culex) pipiens quinquefasciatus* Say 1823 is considered as the most important pests of man and animal in the tropics and has been incriminated as vector of filariasis, which has infected over 120 million people globally, and certain arboviruses in man. It has also been reported as vector of *Dirofilaria immitis* (dog heartworm) and some plasmodia of birds (Becker et al., 2010).

The usage of larvicides still remains as the significant methods among the other methods for mosquito control. The control of mosquito larvae worldwide depends primarily on applications of organophosphates larvicides such as temephos and fenthion (Yang et al., 2002). Unfortunately the side-effects of synthetic organophosphorus compounds on organisms and the environment are increased (Mittal et al., 1991). Recently, botanical insecticides have been applied in mosquito control due to their efficacy, degradability and non-toxic effects on non-target organisms (Dominic Amalraj et al., 2000; Gunasekaran et al., 2004). The usage of plant extracts or essential oils in order to control larval stage of mosquitoes have been investigated by several scientists around the world. Utilizing indigenous plants has shown potential sources of phytochemical with larvicidal activity against vectors of human diseases (Sedaghat et al., 2010, 2011). Latest studies using this approach have revealed larvicidal activity of some indigenous plants from different parts of the world.

The main purpose of this study is to identify of chemical constituents and larvicidal activity of the essential oil of *Kelussia odoratissima* Mozaffarian. This plant is recently described from Iranian flora (Mozaffarian, 2003). *Kelussia (Amirkabiria) odoratissima* is a wild aromatic herb belongs to Apiaceae (Umbelliferae) family with average size 20–60 cm; the well-grown plant can reach 2 m tall. It is native and known from the central region of the Zagros Mountain Chain in Iran. It is a sweet-smelling and is used as a sauce and food in west-central parts of the country. It is also used in traditional medicine to treat hypertension, inflammation, ulcer, cardiovascular diseases and anxiety disorders (Asgary et al., 2004; Ahmadi et al., 2007; Rabbani et al., 2011).

2. Materials and methods

2.1. Plant materials

Fifteen fresh intermediate size (25–30 Cm) of *K. odoratissima* were collected in April 2009 from central Zagros mountain in the Chahar Mahal and Bakhtiari region, Iran (50°13'E, 32°33'N, elevation: 2430 m above sea level). The plant was identified and authenticated and the voucher specimen was deposited at Department of Medical Entomology, Tehran University of Medical Sciences, Tehran, Iran.

2.2. Essential oil isolation

The essential oil of fresh branch tips and leaves (500 g) of *K. odoratissima* was hydro distilled using a clevenger-like apparatus for 3 hours and dried over anhydrous Na₂SO₄. The oil was transferred into an airtight amber-colored vial at 4 °C prior to analysis by gas chromatography–mass spectrometry (GC and GC–MS).

2.3. Gas chromatography–mass spectroscopy

GC analysis was carried out using an HP6890 gas chromatograph equipped with flame ionization detector, an Hp-1capillary

column (30 m × 0.25 mm I.D., film thickness 0.25 µm) and split ratio, 1:25.

The GC settings were programmed as follows: initial oven temperature was held at 40 °C for 1 min, rising to 250 °C at 5 °C/min. The injector temperature was maintained at 250 °C. The detector temperature was at 230 °C. The carrier gas used was helium at a flow rate of 1 ml/min.

GC–MS was performed on Agilent Technology 5973 mass selective detector connected with an HP 6890 gas chromatograph. The oil was analyzed using an HP-1 MS (Fused silica) with the same column and temperature programmed as above. The MS operated at 70 eV ionization energy. Quantitative data were obtained from the electronic integration of the Flame Ionization Detector (FID) peak areas.

2.4. Determination of oil composition

Identification of the oil components was assigned basis on retention indices which were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic conditions. Identification of individual components of the essential oil was performed by computerized matching of their mass spectra and retention indices with Wiley library and those published in the literature (Adams, 2007; Salimi et al., 2010; Rabbani et al., 2011). The percentage of each component is presented in Table 1.

2.5. Mosquito rearing

Fourth instar larvae of *An. stephensi* and *Cx. pipiens* were obtained from Department of Medical Entomology, Tehran University Medical Sciences (TUMS). The colonies were maintained continuously at 28 ± 1 °C with 16:8 light and dark photo period in 65% ± 5% relative humidity. Both larvae of *An. stephensi* and *Cx. pipiens* were continuously available for the mosquito larvicidal experiments.

2.6. Larvicidal bioassay

Larvicidal tests were performed according to the standard method recommended by the World Health Organization (WHO, 1981). All tests were carried out in the test room close to the insectary at temperature 24 ± 1 °C and relative humidity 50% ± 5%. As the essential oil does not dissolve in water it was first dissolved in ethanol 99.0% as co-solvent. Different concentrations of the essential oil in distilled water and the co-solvent were prepared. The oil–ethanol–water solution was stirred for 30 s with a glass rod. For larval test at least 100 larvae of each species were collected by a strainer with fine mesh and then were transferred to a 400-ml glass beaker gently by tapping. Controls included batches of mosquitoes from the colony exposed to water and the solvent alone. The larvae were exposed to different concentrations of 1.25, 2.5, 5, 10 and 20 ppm of essential oil in distilled water for 24 h. The concentrations were chosen to gain the mortality of mosquito larvae between 5% and 95%. Each concentration has been replicated at least 4 times comprising 100 larvae. Logarithmic concentrations indicate that each concentration is a special coefficient of the previous and the next one. For instance, 2.5 ppm is twice of 1.25 ppm and so on. Each glass beaker was left at room temperature and mortality was recorded after 24 h of exposure.

2.7. Statistical analysis

Probit analysis was conducted on mortality data collected after 24 h exposure to different concentration of oil using the method of Finney to determine the lethal concentration for 50% and 90%

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