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Original Article

Chemical Composition and Larvicidal Activity of the Essential Oil of *Laurus nobilis* L. from Iran

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Abstract

The chemical composition of the essential oil obtained from the aerial parts of *Laurus nobilis* L. has been examined by gas chromatography (GC) and GC- mass spectrometery (MS). The main components of the oil were identified. 1,8-Cineole was the major component in the oil together with β-terpinyl acetate, terpinene-4-ol, α-pinene, β-pinene, *p*-cymene, linalool and terpinene-4-ylacetate. The essential oil was tested against *Anopheles stephensi* and *Culex pipiens* larvae. The results obtained show that the essential oil could be considered as natural larvicidal agents.

Keywords: Essential oil; Larvicidal activity; *Laurus nobilis* L.
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1. Introduction

Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria, Japanese yellow fever, dengue and filariasis. The various synthetic products and devices designed to combat such vectors are not successful because of increased resistance developed by various mosquito species. Most of mosquito control programs target the larval stage in their breeding sites with larvicides, because adulticides may only reduce the adult population temporarily [1, 2]. The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent [3].

Plant essential oils in general have been recognized as an important natural resource of insecticides [4, 5]. Their lipophilic nature facilitates them to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects [6]. They have the potential of being acute ovicidal, fumigant, insect growth regulator and insecticid against various insects species [7], and concurrently being developed as ecologically sensitive pesticides [8]. Generally they are safe to humans and other mammals [9, 10].

The present study reports the chemical composition of the essential oil from aerial parts of *Laurus nobilis* L., as well as its larvicidal properties against two mosquitoes species *Anopheles stephensi* and *Culex pipiens* larvae.

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2. Materials and methods

2.1. Plant material

The aerial parts of *Laurus nobilis* L. were collected during its flowering stage in July 2003 from Tabriz (East Azerbaijan province, Iran). A voucher specimen is deposited in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

2.2. Mosquitoes

The third instar larvae of *Anopheles stephensi* and *Culex pipiens* were obtained from laboratory bred culture maintained at ambient rearing conditions. All the bioassays were conducted at 26±1 °C, 60.0±5% RH and 12 h light and 12 h dark photoperiod. Yeast suspension (5%) was used as food source.

2.3. Isolation of the essential oil

Air-dried plant material (100 g) was hydro-distilled for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and then was kept in a sealed vial at 4 °C until analysis.

2.4. Analysis of the essential oil

Gas chromatography (GC) analysis was carried out on a Perkin-Elmer 8500 GC with FID detector and a BP-1 capillary column (30 m×0.25 mm; film thickness 0.25 μm). The carrier gas was helium with a flow rate of 2 ml/min., the oven temperature for first 4 min. was kept at 60 °C and then increased at a rate of 4 °C/min. until reached to the temperature of 280 °C, injector and detector temperature were set at 280 °C.

The mass spectra were recorded on a Hewlett Packard 6890 MS detector coupled with Hewlett Packard 6890 gas chromatograph equipped with HP-5MS capillary column (30 m×0.25 mm; film thickness 0.25 μm). The GC condition was as above. Mass spectrometer condition was as follows: Ionised potential 70 eV, ionisation current 2 A, source temperature 200 °C, resolution 1000, scan time 1 s.

Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature. Quantitative data was obtained from FID area percentages without the use of correction factors [11, 12].

2.5. Larvicidal bioassay

Bioassays were performed according to the WHO protocol [13]. A series of concentrations ranging from 2 to 100 μg/ml of the dissolved oil (in DMSO) was prepared and five replicates were run for each concentration. Control tests were carried out in parallel, using DMSO and water for comparison. Malathion, a conventional insecticide was used as positive control sample. The number of dead larvae were counted after 24 h of exposure and the percentage mortality is reported from the average of five replicates. Observations were also made on the behaviour of larvae.

2.6. Statistical analysis

Probit analysis [14] was conducted on the mortality rate to determine the LC_{50} and LC_{90} representing the concentrations in μg/ml that caused 50% and 90% mortality along with 95% confidence limits

3. Results and discussion

The hydrodistillation of aerial parts of *L. nobilis* gave an oil in 2.1% (w/w) yield, based on the dry weight of the plant that was yellow with distinct sharp odour. Twenty-two components were identified representing 99.5% of total oil. The qualitative and quantitative essential oil composition is presented in Table 1, where compounds are listed in order of their elution on the DB-1 column. The volatile compounds in aerial

Table 1. Essential oil composition of the aerial parts of *Laurus nobilis* L.

Compounds	KI	%
α-thujene	936	0.46
α-pinene	942	5.26
Camphene	953	0.59
Sabinene	972	3.42
β-pinene	976	4.06
α-terpinene	1010	0.50
p-cymene	1013	2.70
1,8-cineole	1021	55.80
γ-terpinene	1048	0.91
Terpinolene	1077	0.35
Linalool	1080	1.40
Pinocarveol	1120	0.48
Pinocarvone	1134	0.35
Terpinene-4-ol	1158	5.27
α-terpineol	1168	0.85
Bornyl acetate	1265	0.76
Terpinene-4-yl acetate	1295	1.13
α-terpinyl acetate	1328	15.14
β-elemene	1382	0.15
β-caryophyllene	1412	0.15
Spathulenol	1558	0.15
Caryophyllene oxide	1564	trace

parts of *L. nobilis* mainly consist of mono- and sesquiterpene hydrocarbons and their oxygenated derivatives. Besides phenolic compounds, also sesquiterpene lactones derived from the germacranolide costunolide can be found. As seen from Table 1, 1,8-cineole is the major component (55.8%), followed by α-terpinyl acetate (15.1 %), terpinene-4-ol (5.3 %), α-pinene (5.2 %), β-pinene (4.0 %), p-cymene (2.7 %), linalool (1.4 %) and terpinene-4-yl-acetate (1.1%). The result of this research is in accordance with other earlier studies on *L. nobilis* that all found to be rich in 1,8-cineole [15, 16].

The essential oil was subjected to laboratory bioassay studies against *A. stephensi* and *C. pipiens* larvae. The tested essential oil demonstrated significant larvicidal activity on both the vector species. Table 2 summarizes the LC_{50} and LC_{90} values for the essential oil. The present study indicated that the essential oil from aerial parts of *L. nobilis* possessed remarkable larvicidal properties and compared favorably with the commercially available insecticide malathion. The results could be useful in search of newer, safer and more effective natural compounds as larvicides. Further studies are needed to devise a formulation using the oil and the compounds of this plant for use as larvicides in mosquito control programs.

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Table 2. Larvicidal activity of essential oil from *Laurus nobilis* against *Anopheles stephensi* and *Culex pipiens*.

Species	LC_{50} (µg/ml)	LC_{90}(µg/ml)	Regression equation	RP
A. stephensi	14.9	22.3	y = 3.17x - 2.69	0.076
C. pipiens	16.5	28.6	y = 3.49x -2.83	0.078

All means are statistically significant ($p< 0.05$).
Numbers in parenthesis are 95% cl values.
RP-Relative potency (LC_{50} standard/LC_{50} test substance).

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