Journal of Applied Horticulture, 12(2): 155-157, July-December, 2010

Chemical composition and larvicidal activity of the essential oil of Iranian *Laurus nobilis* L.

Verdian-rizi Mohammadreza

Department of pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
E-mail: mverdian@razi.tums.ac.ir

Abstract

The chemical composition of the essential oil obtained from the aerial parts of *Laurus nobilis* L., was examined by GC and GC/MS. The main components of the oil were identified. 1,8-cineole was the major component in the oil together with α – terpinyl acetate, terpinene – 4 – ol, α – pinene, β – pinene, p – cymene, linalool and terpinene – 4 – yl – acetate. 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.

Key words: *Laurus nobilis* L., essential oil, hydro distillation, larvicidal activity

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 (El Hag *et al.,* 1999, 2001). 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 (Sukumar *et al.,* 1991).

Plant essential oils, in general, have been recognized as an important natural resource of insecticides (Gbolade *et al.,* 2000; Adebayo *et al.,* 1999). Their lipophilic nature facilitates them to interfere with basic metabolic, biochemical, physiological and behavioural functions of insects (Nishimura, 2001). They have the potential of being acute ovicidal, fumigant, insect growth regulator and insecticidal against various insects species (Tsao *et al.,* 1995) and concurrently being developed as ecologically sensitive pesticides (Isman, 2000). Generally, they are safe to humans and other mammals (Tripathi *et al.,* 2000, 2002).

As a part of our studies on the chemical composition of the essential oils and screening programme for bioactive compounds from plants that grow in Iran, in the present paper, we report the larvicidal activity of the essential oil obtained from the aerial parts of *L. nobilis* L. (Lauraceae) against two species of mosquito vectors, *A. stephensi* and *C. pipiens*. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source.

Materials and methods

Plant material: The aerial parts of *L. nobilis* L. were collected during its flowering stage from Tabriz (East Azerbaijan province, Iran) and identified. A voucher specimen was deposited in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Mosquitoes: The third instar larvae of *A. stephensi* and *C. 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.

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.

Analysis of the essential oil: Gas chromatography analysis was carried out on a Perkin-Elmer 8500 gas chromatograph 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^{-1}, the oven temperature for first 4 min was kept at 60 °C and then increased at a rate of 4 °C min^{-1} 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 gas chromatography condition was as above. Mass spectrometer condition was as follows: ionised potential 70 eV, ionisation current 2A, 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 (Adams, 1995; Dawis, 1990).

Larvicidal bioassay: Bioassays were performed according to the WHO protocol (WHO, 1981). A series of concentrations ranging from 2 to 100 μg mL^{-1} 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.

Statistical analysis: Probit analysis (Raymond *et al.,* 1993) was conducted on the mortality rate to determine the LC_{50} and LC_{90} representing the concentrations in µg mL^{-1} that caused 50% and 90% mortality along with 95% confidence limits.

Results and discussion

The hydrodistillation of aerial parts of *L. nobilis* gave 2.1 % (w/w) oil 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 parts of *L. nobilis* mainly consisted of mono- and sesquiterpene hydrocarbons and their oxygenated derivatives. Besides phenolic compounds, also sesquiterpene lactones derived from the germacranolide costunolide was 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 %). In the present study, the chemical composition of the oil is comparable to that of the previous reports with some variation in the constituents. The observed chemical variations in the composition of the essential oil obtained from the same species are not uncommon. This could be due to different chemotypes for the same species or may result from environmental, developmental or other differences (Hafizoglu *et al.,* 1993; Kilic *et al.,* 2004).

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. 1,8-cineole alone did not show promising activity in the dose re-sponse bioassay against any of the test larvae (mortality >50% was observed only at the highest testdose). Malathion (used as positive control) caused 100% mortality against all the larvae at very low test dose (>0.625 mg L^{-1}).

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

Compounds	RT	KI	Percent
α- thujene	10:00	936	0.46
α- pinene	10:15	942	5.26
camphene	10:42	953	0.59
sabinene	11:27	972	3.42
β- pinene	11:37	976	4.06
α- terpinene	12:59	1010	0.50
p-cymene	13:05	1013	2.70
1,8-cineole	13:25	1021	55.80
γ-terpinene	14:28	1048	0.91
terpinolene	15:36	1077	0.35
linalool	15:45	1080	1.40
pinocarveol	17:15	1120	0.48
pinocarvone	17:49	1134	0.35
terpinene -4- ol	18:42	1158	5.27
α-terpineol	19:05	1168	0.85
bornyl acetate	22:40	1265	0.76
terpinene-4-yl acetate	23:43	1295	1.13
α- terpinyl acetate	24:54	1328	15.14
β- elemene	26:50	1382	0.15
β- caryophyllene	27:52	1412	0.15
spathulenol	32:34	1558	0.15
caryophyllene oxide	32:47	1564	Trace

Table 2. Larvicidal activity of essential oil from *L. nobilis* L. against *A. stephensi* and *C. pipiens*

Species	LC_{50} (µg mL^{-1})	LC_{90} (µg mL^{-1})	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 ($P < 0.05$).
RP—Relative potency (LC_{50} standard/LC_{50} test substance).

The present study indicated that the essential oil from aerial parts of *L. nobilis* L. possessed remarkable larvicidal properties and compared favourably with the commercially available insecticide malathion. Larvicidal activity of 1,8-cineole, a major constituent (55.80 %) in the *L. nobilis* L. essential oil, was also studied to compare its activity with that of the *L. nobilis* L. oil. Surprisingly, this compound when tested alone failed to produce promising activity against any of the mosquito larvae (mortality >50% was observed only at the higher test dosages). In the present study, besides 1,8-cineole there are many other oxygenated monoterpenes and related compounds present in the oil. Thus, the activity of the oil against the mosquito larvae may be attributed to the additive or synergistic or blend effect of many or some of the constituents. Such an effect has been previously observed with some essential oils where the activity was due to the combination of the major constituents, none of which was found to exhibit significant activity, individually (Omolo *et al.,* 2005).

Recently, promising larvicidal activities of many essential oils and their compositions against mosquito vectors have reemphasised the need to explore the possibility of using essential oil-based products as supplementary and complimentary measures for mosquito borne diseases. 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.

Acknowledgement

We are grateful to the Faculty of Pharmacy, Tehran University of Medical Sciences, for the financial support to this investigation.

References

Adams, R.P. 1995. *Identification of Essential Oils by Ion Trap Mass Spectroscopy.* Academic Press: San Diago.

Adebayo, A., A.A. Gbolade and J.I. Olaifa, 1999. Comparative study of toxicity of essential oils to larvae of three mosquito species. *Nig. J. Nat. Prod. Med.*, 3: 74-76.

Dawis, N.W. 1990. Gas chromatography retention indices of monoterpenes and sesquiterpenes on methyl silicon and carbowax 20 M phases. *J. Chromat.,* 503: 1-24.

El-Hag, E.A., A.H. Nadi and A.A. Zaitoo, 1999. Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). *Phytother. Res.*, 13: 388-92.

El-Hag, E.A., A. Rahman, H. El-Nadi and A.A. Zaitoo, 2001. Effects of methanolic extracts of neem seeds on egg hatchability and larval development of *Culex pipiens* mosquitoes. *Ind. Vet. J.*, 78: 199-201.

Gbolade, A.A., A.O. Oyedele, M.B. Sosan, F.B. Adewayin and O.L. Soyela, 2000. Mosquito repellent activities of essential oils from two Nigerian *Ocimum* species. *J. Trop. Med. Plants*, 1: 146-148.

Hafizoglu, H. and M. Reunanen, 1993. Studies on the components of *Laurus nobilis* from Turkey with special references to laurel berry fat. *Fat Sci. Technol.*, 95: 304-308.

Isman, M.B., 2000. Plant essential oils for pest and disease management. *Crop Prot*., 19: 603-608.

Kilic, A., H. Hafizoglu, H. Kollmannsberger and S. Nitz, 2004. Volatile constituents and key odorants in leaves, buds, flowers, and fruits of *Laurus nobilis* L. *J. Agric. Food Chem*., 52: 1601-1606.

Nishimura, H. 2001. Aroma constituents in plants and their repellent activities against mosquitoes. *Aroma Res*., 2: 257-267.

Omolo, M.O., D. Okinyo, I.O. Ndiege, W. Lwande and A. Hassanali, 2005. Fumigant toxicity of the essential oils of some African plants against *Anopheles gambiae sensu stricto*. *Phytomedicine,* 12: 241-6.

Raymond, M., G. Prato and D. Ratsira, 1993. PROBIT Analysis of Mortality Assays Displaying Quantal Response, Version 3.3 Licence L93019, Praxeme, 34680 St. Georges d'Orques, France.

Sukumar, K., M.J. Perich and L.R. Boobar, 1991. Botanical derivatives in mosquito control. A review. *J. Am. Mosq. Control Assoc.*, 72: 210-237.

Tripathi, A.K., V. Prajapati, K.K. Aggarwal, S.P.S. Khanuja and S. Kumar, 2000. Repellency and toxicity of oil from *Artemisia annua* to certain stored product beetles. *J. Econ. Entomol*., 93: 43-47.

Tripathi, A.K., V. Prajapati, N. Verma, J.R. Bahl, R.P. Bansal and S.P.S. Khanuja, 2002. Bioactivities of the leaf essential oil of *Curcuma longa* (var. CH-66) on three species of stored-product beetles (Coleoptera). *J. Econ. Entomol*., 95: 183-189.

Tsao, R., S. Lee, P.J. Rice, C. Jensen and J.R. Coats, 1995. Monoterpenoids and their synthetic derivatives as leads for new insect control agents. In: *Synthetic and Chemistry of Agrochemicals*. Baker, D.R., J.G. Fenyes and G.S. Basarab(Eds.), IV, American Chemical Society, Washington, DC., pp: 312-324.

World Health Organization, 1981. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticide. WHO/VBC/81. 807.

Received: November, 2009; Revised: June, 2010; Accepted: July, 2010