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Chemical Constituents and Larvicidal Activity of the Essential Oil of Polylophium Involvucratum (Pall.) Boiss (Apiaceae)

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Abstract: The chemical composition of the essential oil obtained from the seeds of *Polylophium involvucratum* (pall.) Boiss has been examined by GC and GC/MS. The main components of the oil has been identified. The major components were limonene (60.39%), aldehyde and alcohol derivatives of limonene (34.45%). The laboratory bioassay of the essential oil against *Anopheles stephensi* and *Culex pipiens* was carried out to evaluate the larvicidal activity. The tested oil proved to have strong larvicidal activity against *A. stephensi* larva (LD_{50}: 8.01-13.59 mg mL^{-1}) and *C. pipiens* larva (LD_{50}: 3.68-5.13 mg mL^{-1}).

Key words: *Polylophium involvucratum*, essential oil, limonene, *Anopheles stephensi*, *Culex pipiens*

INTRODUCTION

Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria, Japanese encephalitis, 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 the mosquito control programmes 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 programme 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 *et al*., 2001). They have the potential of being acute ovicidal, fumigant, insect growth regulator and insecticidal against various insect species (Tsao *et al*., 1995) and concurrently being developed as ecologically sensitive pesticides (Isman *et al*., 2000). Generally they are safe to humans and other mammals (Tripathi *et al*., 2000, 2002).

The present study reports systematic investigation on chemical constituents and the larvicidal effects of essential oil of *Polylophium invovucratum* (pall.) Boiss against two mosquito species, *Anopheles stephensi* and *Culex pipiens*.

MATERIALS AND METHODS

Plant Materials

Polylophium involvucratum (pall.) Bioss was collected from the north of Iran in June 2000. The plant sample was identified and voucher specimen deposited at the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences with the herbarium No. of 7850.

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Essential Oil Distillation

The essential oil was obtained by hydrodistillation of seeds (100 g) in a Clevenger type apparatus for 3 h. The resulting essential oil was 3.8% v/w of the seeds.

Analysis of the Essential Oil

The oil was analyzed on a Varian CP-3380 gas chromatograph with flame ionization detectors fitted with a fused silica capillary column (30 m × 0.25 mm i.d.) coated with DB1 phase, film thickness 0.25 μm; temperature program 50-200°C at 5°C min^{-1}, injector temperature 220°C, detector temperature 250°C, carrier gas N_2 at 0.8 mL min^{-1}. GC/MS analyses were performed using a Hewlett-Packard apparatus equipped with a HP_1 fused silica column (30 m × 0.25 mm; film thickness 0.25 μm) and interfaced with a quadrupole detector (Model 5970). Column temperature was programmed from 70-200°C at 10°C min^{-1}; injector temperature was 220°C. Helium was used as carrier gas at a flow rate of 0.6 mL min^{-1}; the mass spectrometer was operated at 70 eV. The identification of the constituents was assigned on the basis of comparison of their retention indices (Kovats indices) and their mass spectra with those given in the literature (McLafferty and Stauffer, 1989; Adams, 1995; Joulain and König, 1998).

Mosquito Larvicidal Test

The essential oil was evaluated at the level of 2.5, 5, 10, 20 and 40 mg mL^{-1} in tap water. Tween-80 was used as emulsifier at a concentration of 0.001%. Tap water mixed with Tween-80 was used as control. Standard WHO test (WHO, 1981) was employed with slight modification in the test procedure. A single fourth-stage larva was put into each of 20 vials containing 5.0 mL of the test solution of each concentration. Observation on larval mortality was recorded after 10, 20 and 30 min. Larvae were considered dead, when they did not react to touching with a needle. Mortality data were subjected to probit analysis to analyze lethal doses (LD_{50}). Malathion, a conventional insecticide was used as positive control sample.

RESULTS AND DISCUSSION

Hydrodistillation of the dried plant material, followed by extraction with diethyl ether and careful evaporation at low temperature produced light blue oil with a yield of 3.8% (by weight). The identity, retention time and the percentage composition of the oil of *Polylophium invovucratum* (pall.) Boiss are presented in Table 1. The percentage composition is presented as relative peak area. Four components were identified and quantified, accounting for over 99% of the composition of the oil. None of the remaining unidentified compounds accounted for more than 0.7% of the total peak area. The major components were limonene (60.39%), aldehyde and alcohol derivatives of limonene (34.45%).

LD_{50} of the essential oil was 8.01, 10.74 and 13.59 mg mL^{-1} on *Anopheles stephensi* larva and 3.68, 4.75 and 5.13 mg mL^{-1} on *Culex pipiens* larva after 10, 20 and 30 min, respectively (Table 2 and 3). The essential oil has more activity against *Culex pipiens* larva than *Anopheles stephensi* larva. Earlier authors have reported larvicidal activity of essential oils of various medicinal and aromatic plants and isolated compounds against the larva of *Anopheles stephensi* and *Culex pipiens* (Ansari *et al*., 2000; Ezeonu *et al*., 2001; Sen-Sung *et al*., 2003; Thomas *et al*., 2004).

Table 1: Chemical composition of *Polylophium invovucratum* (pall.) boiss essential oil

Identification	R.t (sec)	Percentage of total oil
Perillaldehyde	837	25.80
α-Pinene	938	7.15
Perillalcohol	940	6.65
Limonene	942	60.39

Table 2: Larvicidal activity of *Polylophium invovucratum* (pall.) boiss essential oil against *Anopheles stephensi*

A	b±SE	Time (min)	LD_{50}±95% CI
-35	75.0±0.1240	10	8.01
-33.1	80.6±0.2750	20	10.74
-20.3	77.78±0.264	30	13.59

A = Intercept, b±SE = Slope±standard error, LD_{50}±95% CI = Lethal dose cause 50% mortality, 95% confidence interval

Table 3: Larvicidal activity of *Polylophium invovucratum* (pall.) boiss essential oil against *Culex pipiens*

A	b±SE	Time (min)	LD_{50}±95% CI
-4.04	70.8±0.293	10	3.68
-11.11	90.3±0.143	20	4.75
4.4	80.6±0.234	30	5.13

A = Intercept, b±SE = Slope±standard error, LD_{50}±95% CI = Lethal dose cause 50% mortality, 95% confidence interval

The findings of the present investigation indicate larvicidal properties in the essential oil of *Polylophium invovucratum* (pall.) Boiss against two mosquito species. Although the specific components of the studied essential oil that elicit anti-plasmodial activity remain unclear, present data suggest that the oil offers new possibilities for antimalarial chemotherapy. Although essential oils are necessarily difficult to study and difficult to utilize as drugs, they may offer a unique means of discovering new effective antimalarial drugs from plants with medicinal uses in endemic countries. Important goals will be to identify the active components of essential oils with antimalarial activity and to determine the mechanisms by which these compounds exert their biological activities.

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