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RESEARCH ARTICLE

Larvicidal effects of the major essential oil of *Pittosporum tobira* against *Aedes aegypti* (L.)

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Abstract

Essential oil obtained from the leaves of *Pittosporum tobira* was extracted and its chemical composition and larvicidal effects were studied. Analyses were conducted by gas chromatography and mass spectroscopy (GC-MS) to determine the primary constituents of the essential oil of *P. tobira*. The yield of *P. tobira* essential oil (PTEO) was 0.1%, and GC-MS analysis identified its major constituents as undecane (31.11%), 4-methyl-1,3-pentadiene (11.34%), (1,3-dimethyl-2-butenyl)benzene (5.45%), and L-limonene (14.08%). The essential oil had a significant toxic effect against early fourth-stage larvae of *Aedes aegypti* (L.), with an LC_{50} value of 58.92 ppm and an LC_{90} value of 111.31 ppm. Finally, the LC_{50} and LC_{90} values of L-limonene were 39.7 ppm and 78.11 ppm. These results could be useful for seeking newer, safer, and more effective natural larvicidal agents against *A. aegypti*.

Keywords: Aedes aegypti*; essential oils; larvicidal effects;* Pittosporum tobira*; l-limonene*

Introduction

Synthetic insecticides have created a number of ecological problems, such as the development of resistant insect strains, ecological imbalance, and harm to mammals. Natural products are generally preferred because of their less harmful nature to nontarget organisms and due to their innate biodegradability[1]. The mosquito *Aedes aegypti* is the important vector of dengue fever and dengue hemorrhagic fever in many parts of the world. In the absence of effective vaccine and drugs, dengue prevention and control programs have depended on vector control. Management of this disease vector using synthetic organic chemical insecticides has failed because of insecticide resistance developed by the *Aedes* mosquitoes[2]. In the search for environmentally safe and relatively inexpensive methods for controlling mosquitoes, plant extracts have received much interest as potential bioactive agents against mosquito larvae. Most of the mosquito control programs target the larval stage in their breeding sites with larvicides[3], because the adulticides may only reduce the adult population temporarily[4]. Therefore, a more efficient way to reduce the mosquito population is to target the larvae.

Pittosporum tobira (Pittosporaceae) is a small, slender, evergreen tree that grows on the southwestern Pacific coast of Jeiju Island. The seeds undergo a gradual change in color from green to red in late autumn to winter[5]. Nickavar *et al.* reported the volatile components of the flower and fruit oils from *P. tobira* grown in Iran, obtained through hydrodistillation and analyzed by gas chromatography-mass spectroscopy (GC-MS)[6]. Rodrigues Frederico *et al.* reported the volatile components of the leaf, flower, and fruit volatile oils of *P. tobira* grown in three locations in Portugal[7]. The essential oil from *P. tobira* (leaves) shows that target sites other than those used by antibiotics will be active against multidrug-resistant microbial pathogens. However, very little information is available on such activity of aromatic herbs[8]. Maoka *et al.* reported the isolation and structural elucidation of novel carotenoids from the seeds of *P. tobira* grown in Japan[9,10]. Fujiwara *et al.* reported the isolation and structural elucidation of new carotenoids from the seeds of *P. tobira*[11–13].

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D'Acquarica *et al*. reported the isolation and structural elucidation of four new triterpenoid estersaponins from the fruits of *P. tobira*[14]. Takaoka *et al*. reported the structures of sesquiterpene glycosides from *P. tobira*[15]. Nozaki *et al*. reported the structures of three new germacranolide glycosides from *P. tobira*[16]. Ogihara *et al*. reported the structures of sesquiterpene glycosides and other terpene constituents from the flowers of *P. tobira*[17]. Suga *et al*. reported the structure of a new sesquiterpene glycoside from the flowers of *P. tobira*[18]. However, the biological activity of *P. tobira* has not been investigated in detail.

The aim of this study was to investigate the primary chemical composition and larvicidal properties of the essential oil of *P. tobira*. To the best of our knowledge, this is the first report on the major chemical composition and larvicidal activity of the essential oil of *P. tobira*.

Materials and methods

Plant materials and essential oil extraction

Fresh leaves of *P. tobira* were collected in July 2004 at Jeiju Island (South Korea) and subjected to hydrodistillation using a Clevenger-type apparatus for 6 h. The *P. tobira* essential oil (PTEO) was dried over anhydrous sodium sulfate, and the purified essential oil was stored in an amber-colored vial at 4°C until further use.

Chemicals

L-limonene (≥95.0%), 4-methyl-1,3-pentadiene (≥98.0%), and undecane (≥99.8%) were purchased from Sigma-Aldrich Korea (Yongin City, Kyunggi-Do, South Korea).

Gas chromatography-mass spectroscopy analysis of the essential oil

GC-MS analysis of the essential oil was performed using a GC-MS spectrometer (QP 2010; Shimadzu, Kyoto, Japan), equipped with a splitless injector. The components were separated on a 0.32 mm i.d. × 60 m DB-1 MS capillary column (Agilent Scientific, Santa Clara, CA, USA) with a film thickness of 0.25 µm. The temperature of the injector was set at 300°C. The initial temperature was set at 80°C and held for 5 min, increased by 5.0°C/min to 280°C, and then held for 10 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. One microliter of the sample (diluted 1:10 with acetone) was injected with a split ratio of 1:100. The percent composition of the essential oil was calculated by comparing areas of the GC peaks. The temperatures of the ion source and the injector were set at 200°C and 210°C, respectively. The interface was maintained at 280°C, and mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The sector mass analyzer was set to scan from 50 to 500 amu every 0.5 s. The various components of the essential oil were identified by comparing the mass spectrum of each peak with those of authentic samples found in a library of mass spectra (*Wiley Registry of Mass Spectral Data*, 7th ed.).

Larvicidal assay

The F^{21} laboratory strain of *A. aegypti* Linn was obtained in 2008 from the National Institute of Health, Seoul, South Korea. Adult female mosquitoes were maintained on a 10% sucrose solution, and anesthetized mice were used for blood feeding of the mosquitoes. Larvae were reared in plastic trays and fed a diet of chicken chow and yeast (8:2). Mosquitoes were maintained at 27 ± 2°C, 70 ± 5% relative humidity, and a photoperiod of 16L:8D. The larvicidal activity was analyzed according to the standard procedures recommended by the World Health Organization[19]. The essential oil was dissolved in 1 mL acetone, and various concentrations were prepared (0, 12.5, 25, 50, 100, and 200 ppm) using distilled water. Twenty larvae at the early fourth stage were used in the larvicidal assay, and five replicates were maintained for each concentration. Larval mortality was calculated after 24 h of exposure. The lethal concentrations LC_{50} and LC_{90} and 95% confidence intervals were calculated using profit analysis (SigmaPlot® software; Systat Software, Inc., San Jose, CA, USA).

Results and discussion

The *Pittosporum tobira* leaves yielded 0.1% (v/w) essential oil with a foul odor. Table 1 lists the major chemical constituents, as identified by GC and GC-MS analyses. In order of elution from the column, these major compounds were undecane (31.11%), 4-methyl-1,3-pentadiene (11.34%), (1,3-dimethyl-2-butenyl)benzene (5.45%), and L-limonene (14.08%). The larvicidal effects of PTEO are presented in Table 2. The oil had significant toxic effects against the larvae of *A. aegypti*, with an LC_{50} value of 58.92 ppm and an LC_{90} value of 111.31 ppm. Also, L-limonene (≥95.0%), 4-methyl-1,3-pentadiene (≥98.0%), and undecane (≥99.8%) were tested against the F^{21} laboratory strain of *A. aegypti*. The current *Pittosporum* species was tested for the first time against *A. aegypti*, and L-limonene (≥95.0%), 4-methyl-1,3-pentadiene (≥98.0%), and undecane (≥99.8%) were tested for the first time against mosquito larvae. L-limonene was the most toxic among the three major components (L-limonene, 4-methyl-1,3-pentadiene, and undecane), with an LC_{50} value near 39.7 ppm. The above indicates that L-limonene may play an important role in the toxicity of essential oil. The larvicidal effects are summarized in Tables 2 and 3. In general, plant essential oils have been recognized as important natural sources of insecticides[20,21]. Differences in the toxicity of essential oils against various

Table 1. Major constituents of *Pittosporum tobira* essential oil.

Retention time[a]	Component	M^+	Peak area (%)	Fragments	
1.13	Undecane	156	31.11	57	43
1.27	L-limonene	136	14.08	93	69
1.56	4-Methyl-1,3-pentadiene	82	11.34	67	43
1.84	(1,3-Dimethyl-2-butenyl) benzene	160	5.45	91	105

[a]Retention time relative to that of α-pinene.

Table 2. Larvicidal activity of *Pittosporum tobira* essential oil against *Aedes aegypti*.

Concentration (ppm)	Percent mortality ± SE	LC_{50} (ppm)	LC_{90} (ppm)	95% Confidence interval for LC_{50}	95% Confidence interval for LC_{90}
12.5	13 ± 2.1				
25	28 ± 4.5				
50	49 ± 7.3	58.92	111.31	33.23–89.24	94.16–201.98
100	88 ± 4.9				
200	95 ± 7.9				

Table 3. LC_{50} and LC_{90} values for undecane, L-limonene, and 4-methyl-1,3-pentadiene.

Compound	LC_{50} (ppm)	LC_{90} (ppm)	95% Confidence interval for LC_{50}	95% Confidence interval for LC_{90}
Undecane	NE	NE	NE	NE
L-limonene	39.7	78.11	30.28–40.74	62.34–105.26
4-Methyl-1,3-pentadiene	76.2	109.33	61.34–103.11	83.29–200.24

Note. NE, not effective.

mosquito species have been well-documented[22], and arise because of qualitative and quantitative variations in their components. For example, Cheng *et al*.[23] reported the larvicidal activity of linalool from *Cinnamomum osmophloeum* against *A. aegypti* (LC_{50} = 50 ppm).

The findings of the present study indicate that essential oil extracted from the leaves of *P. tobira* could be studied as a potential natural larvicide.

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