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Chemical Composition and Acaricidal Effects of Essential Oils Extracted from *Ligustrum japonicum* against Acaridae and Pyroglyphid Mites

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Abstract The composition of the essential oil of *Ligustrum japonicum* leaves was determined by GC-MS analysis. The major constituents of *L. japonicum* leaf oil were germacrene D (40.50%), α-pinene (13.63%), (−)-β-elemene (6.42%), β-caryophyllene (5.73%), and δ-cadinene (5.47%). The acaricidal activities of *L. japonicum* oil were evaluated against acaridae and pyroglyphid mites. In the fumigant bioassay, the LD_{50} values of *L. japonicum* oil were 16.48, 12.38, and 15.63 μg/cm^3 against *Tyrophagus putrescentiae*, *Dermatophagoides farinae*, and *D. pteronyssinus*, respectively. In the contact bioassay, the LD_{50} values of *L. japonicum* oil were 8.02, 5.02, and 7.67 μg/cm^2 against *T. putrescentiae*, *D. farinae*, and *D. pteronyssinus*, respectively.

Keywords acaricidal activity · *Ligustrum japonicum* · pyroglyphid mites · *Tyrophagus putrescentiae*

The significance of acaridae mite, *Tyrophagus putrescentiae* (storage mite), and pyroglyphid mites, *Dermatophagoides farinae* and *D. pteronyssinus*, with regard to allergic diseases is known in the world (Arlian, 1989). These mites are the most serious factors of allergens, causing asthma and atopic dermatitis (Yang and Lee, 2012). Acaridae and pyroglyphid mites have been known to be the hygienic pests for storage products and apartment dusts and are important allergen factors within stored grains and apartment environments in the humid areas (Azima et al., 2011). Stored grains, wall-to-wall carpeting, and space heating in the lifestyle of humans have promoted favorable environmental conditions for the growth of acaridae and pyroglyphid mites (Yang et al., 2013). The population of acaridae and pyroglyphid mites has preferentially been controlled by synthetic acaricide such as *N*,*N*-diethyl-*m*-toluamide (DEET). However, repeated attempts of synthetic acaricides have resulted in residual toxicity and resistance (Jeon and Lee, 2011). These problems have pointed the need for the development of effective acaricides against acaridae and pyroglyphid mites (Yang et al., 2014).

Ligustrum japonicum Thunb. (Oleaceae) is known as Wax Privet originating from Korea and Japan. This tree has a thick covering of green leaves throughout the whole year, making it an evergreen plant. The *L. japonicum* flowers are followed by black fruits in June. The fruit is an oval drupe ripening in purple-black color with a glaucous bloom in December. Previous studies have indicated that *L. japonicum* flowers are abundant in active materials responsible for aldose reductase inhibition (Papoti et al., 2011), antibacterial (Doh, 2010), and antioxidant (Papoti et al., 2011) effects. It contains α-terpinolene, hotrienol, 2-methoxyphenol, benzenemethanol, and tricosane (Lim et al., 2008). However, there are a few studies on the acaricidal effects of the *L. japonicum*-derived materials against acaridae and pyroglyphid mites. To analyze the composition of the essential oils obtained from *L. japonicum* leaves, the volatile constituents of *L. japonicum* oil were identified by GC-MS to assess the acaricidal effects.

Sample preparation and GC-MS analysis. *L. japonicum* leaves were purchased from Youngchon local market (Korea) and extracted by steam distillation. The essential oil obtained from the extract (yield 0.18%) was dried over Na_2SO_4 and concentrated by evaporation (EYELA NAJ-100, Japan) at 31°C. *L. japonicum* oil was analyzed by GC-MS (5973 IV, Agilent, USA). DB-5 column (30 m×0.25 mm, Folsom, CA) was used for GC-MS analysis, and the column conditions used are as follows: injector temperature, 210°C; DB-5 column temperature, isothermal at 51°C for 16 min, then increased to 203°C at 1.7°C/min and maintained at this

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temperature for 16 min; an ion source temperature, 231°C. Helium was injected at a rate of 0.7 mL/min. The effluent of the DB-5 column was used in the mass spectrometer. Spectra were measured in the electron ionization mode at 71 eV. The mass was analyzed in the *m/z* range between 10 and 425, and the sector mass analyzer was set in the scan range 50-800 amu for 2 s. The chemical composition was determined by comparing the retention time, and mass data was obtained when the standard chemical was analyzed using the DB-5 column. When the standard chemical was unavailable, the identification of the compounds derived from *L. japonicum* oil was measured by comparing The Wiley Registry of Mass Spectral Data (7th ed.) and Adams library (Adams, 2007).

Acaridae and pyroglyphid mites. The cultures of *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* were kept in an incubator. These mites were reared in the containers (25×25×20 cm^3) containing diet (dried yeast and fry feed no. 1) at 26±2°C and 69±4% relative humidity (RH) in dark area. The fry feed consisted of cellulose (4.0%), calcium (1.8%), protein (44.0%), phosphate (2.0%), lipids (3.0%), and others (40.2%). To synchronize the mite developmental cycles, acaridae and pyroglyphid mites were placed in dishes (80×20 mm^2) and allowed to lay eggs for 50 h. The laid eggs of acaridae and pyroglyphid mites were checked for the stages using a microscope until adults died. The fumigant method was modified by the bioassay described by Lee et al. (2009). The acaricidal activities of *L. japonicum* oil were bioassayed using the fumigant bioassay against acaridae and pyroglyphid mites. Different quantities (20–10 μg/cm^3) of *L. japonicum* oil were dissolved in acetone (25 μL) and applied to disks (0.8×0.1 cm^2). Pure acetone was used as the negative control. The treated paper disk was dried in a hood for 10 min and then placed at the top of a microtube (2 mL). Thirty acaridae and pyroglyphid mites were inoculated in microtubes, which were sealed using the cap with the paper disk. Biological comparisons and processing of the samples were determined under the same conditions as acaridae and pyroglyphid mites for 24 h. The acaricidal effects of the samples were measured using the contact bioassay against acaridae and pyroglyphid mites by the acaricidal bioassay modified as described by Jeon et al. (2012). Six concentrations (10–3 μg/cm^2) of *L. japonicum* oil were liquefied in acetone (50 μL). The sample was injected in a petri dish (35×10 mm^2) and dried for 10 min. Thirty acaridae and pyroglyphid mites were inoculated in petri dishes, and the lid was closed. The dishes were maintained at 25±1°C and 70% RH for 24 h. Mite mortalities were measured using a microscope after 24 h. All the treatments were replicated twice, and LD_{50} was measured using SAS software (SAS Institute, 1990). The relative toxicity was calculated based on DEET LD_{50}/sample LD_{50} (Jeon et al., 2012).

L. japonicum oil was measured by GC-MS, and retention time, retention index, and mass spectra of each constituent were compared to those reported in the literature (Hendriks et al., 2005; Lim et al., 2008). The chemical composition of the essential oil is listed in Table 1. Relative composition (%) of the constituents derived from *L. japonicum* oil was germacrene D (40.50%), α-pinene (13.63%), (−)-β-elemene (6.42%), β-caryophyllene (5.73%), δ-cadinene (5.47%), α-humulene (4.46%), α-copaene (4.33%), α-cadinol (3.66%), (±)-limonene (2.91%), muurolol (2.89%), α-muurolene (2.76%), β-pinene (2.34%), (+)-cycloisosativene (1.55%), myrcene (0.88%), α-amorphene (0.68%), α-terpineol (0.34%), and (−)-borneol (0.31%). The constituents of *L. japonicum* oil were grouped as alkenes (β-caryophyllene, α-copaene, (+)-cycloisosativene, (−)-β-elemene, germacrene D, α-humulene, (±)-limonene, myrcene, α-pinene, and β-pinene), alcohol ((−)-borneol, α-cadinol, muurolol, and α-terpineol), and naphthalene (α-amorphene, α-muurolene, and δ-cadinene). According to Lim et al. (2008), the volatile components of essential oil extracted from *L. japonicum* flowers were α-terpinolene (4.36%), hotrienol (9.21%), 2-methoxyphenol (5.01%), benzenemethanol (6.72%), tricosane (8.05%), and 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol (5.20%). In the current and previous studies, the volatile constituents of the essential oils derived from *L. japonicum* flowers and leaves were affected by the harvest time, storage period, intraspecific variability, handling method, and experimental methods, indicating the optimum extraction method and plant parts (flowers, leaves, and fruits) (Hendriks et al., 2005).

Table 1 GC-MS analyses of volatile constituents from *L. japonicum* oil

Retention time	Constituents	Relative composition (%)
4.946	α-Pinene	13.63
5.746	β-Pinene	2.34
5.921	Myrcene	0.88
6.688	(±)-Limonene	2.91
9.220	(−)-Borneol	0.31
9.612	α-Terpineol	0.34
12.518	(+)-Cycloisosativene	1.55
12.616	α-Copaene	4.33
12.814	(−)-β-Elemene	6.42
13.295	β-Caryophyllene	5.73
13.773	α-Humulene	4.46
14.171	Germacrene D	40.50
14.338	α-Muurolene	2.76
14.552	α-Amorphene	0.68
14.642	δ-Cadinene	5.47
16.195	Muurolol	2.89
16.359	α-Cadinol	3.66

Essential oil was extracted from *L. japonicum* leaves in 0.18% yield (v/w). To measure the acaricidal effects, the acaricidal potential of *L. japonicum* oil was determined by the contact and fumigant bioassays against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*. Compared to the LD_{50} values of DEET, the acaricidal potential of *L. japonicum* oil (12.38, 15.63, and 16.48 μg/cm^3) proved to be more toxic than that of DEET (36.50, 34.23, and 30.31 μg/cm^3) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* in fumigant bioassay, respectively (Table 2). In the contact bioassay, the acaricidal activity of *L. japonicum* oil (5.02, 7.67, and 8.02 μg/cm^2) was more potent than that of DEET (19.64, 14.12, and 14.15 μg/cm^2) against *D. farinae*, *D. pteronyssinus*,

Table 2 Acaricidal effects of *L. japonicum* oil and a synthetic acaricide against acaridae and pyroglyphid mites[a]

Samples	Bioassay	Mite species	LD_{50}	95% CL	RT[b]
L. japonicum oil	Fumigant (μg/cm^3)	*D. farinae*	12.38	11.39–13.37	2.95
		D. pteronyssinus	15.63	14.64–16.62	2.19
		T. putrescentiae	16.48	15.45–17.38	1.84
	Contact (μg/cm^2)	*D. farinae*	5.02	4.78–5.26	3.91
		D. pteronyssinus	7.67	6.92–8.42	1.84
		T. putrescentiae	8.02	7.14–8.81	1.76
DEET	Fumigant (μg/cm^3)	*D. farinae*	36.50	36.44–36.55	1.00
		D. pteronyssinus	34.23	34.18–34.29	1.00
		T. putrescentiae	30.31	30.22–30.39	1.00
	Contact (μg/cm^2)	*D. farinae*	19.64	19.63–19.66	1.00
		D. pteronyssinus	14.12	14.11–14.13	1.00
		T. putrescentiae	14.15	14.02–14.26	1.00

[a]Exposed for 24 h.
[b]RT, Relative toxicity=LD_{50} value of DEET/LD_{50} value of each compound.

and *T. putrescentiae*, respectively (Table 2), indicating that *D. farinae* is more susceptible to *L. japonicum* oil than *D. pteronyssinus* and *T. putrescentiae,* regardless of the bioassays. The acaricidal potential of *L. japonicum* oil depends on the bioassay conditions and mite species (Oh et al., 2012). Moreover, the acaricidal activities of *L. japonicum* oil against three acaridae and pyroglyphid mites were attributed to borneol and β-caryophyllene. The acaricidal activities of borneol and β-caryophyllene against three acaridae and pyroglyphid mites have been reported previously (Lee et al., 2010; Oh et al., 2014). Nevertheless, this study is the first report on the acaricidal effects of *L. japonicum* oil against three acaridae and pyroglyphid mites. In conclusion, our study showed that the essential oil of *L. japonicum* leaves may be useful to develop an ecofriendly agent for managing three acaridae and pyroglyphid mites.

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