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Bioactivity Investigation of Lauraceae Trees Grown in Taiwan

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Abstract

This research collected 27 Lauraceae tree species in Taiwan, and the extracts prepared from leaves and branches were selected to evaluate and characterize their putative bioactivities and potential medicinal applications. Several bioactivity assays, including antifungal tests, antioxidant evaluation, anti-inflammation activity, and cytotoxicity were performed in this study. The results showed no significant antifungal activity by Lauraceae extracts. *Neolitsea parvigemma* (Hay.) Kanehira et Sasaki expresses the best antioxidant activity ($IC_{50} = 5.73 \mu\text{g/mL}$) in the DPPH assay. The extracts of *Litsea akoensis* Hay. and *Cryptocarya concinna* Hance had significant anti-inflammation activity, and they can inhibit the nitric oxide (NO) production in the LPS-induced microphage assay at the dose of $25 \mu\text{g/mL}$. According to the cytotoxicity assay, *Lindera aggregate* (Sims) Kosterm and *Cryptocarya concinna* Hance extracts showed *in vitro* cytotoxicity against human umbilical vein endothelial cell line (HUVEC) with IC_{50} values of $43.15 \mu\text{g/mL}$ and $49.36 \mu\text{g/mL}$, respectively, and *Phoebe formosana* (Matsum. et Hay.) Hay. extract exhibited marked cytotoxicity ($IC_{50} = 42.87 \mu\text{g/mL}$) against a human leukemia cell line (HL-60). Results from this preliminary investigation suggest that these Lauraceae tree species may have a great potential for further development as cancer chemoprevention agents or food supplements for promoting human health.

Keywords: Antifungal, anti-inflammation, antioxidant, bioactivity, cytotoxicity, Lauraceae.

Introduction

There are more than 2500 species belonging to the Lauraceae family all over the world, distributed within the subtropics and tropics of eastern Asia and South and North America (Simie et al., 2004). Many plants of Lauraceae have been employed in folk medicine for their interesting bioactivities. For example, *Cinnamomum camphora* (L.) Presl is a major source of camphor, which can be made into camphor oil and mothballs. In addition, camphor is taken orally to calm hysteria, nervousness, neuralgia, and to treat serious diarrhea. Camphor is also known to be effective in treating colds and chills (Lee et al., 2006). The bark of *Cinnamomum cassia* Blume is a very famous traditional medicine that has been widely used in Asian countries. The extracts from *C. cassia* have been claimed to reduce inflammation (Lee & Shibamoto, 2002), and to decrease serum glucose, total cholesterol, and platelet counts (Khan et al., 2003).

Owing to its unique ecosystem, Taiwan is famous for the abundance and diversity of its flora, with more than 4500 plant species classified to date. In Taiwan, Lauraceae is an economically important family, consisting mostly of trees, and growing throughout the island, from the lowlands up to an altitude of 1500 m (Liao, 1996). There are around 60 Lauraceae tree species grown in Taiwan. Although there are some studies that focus on bioactivity investigations of Lauraceae grown in Taiwan (Table 1), systematic collection and bioactivities screening are still worthy of further investigation. On the other hand, from a natural conservation point of view, the

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Table 1. Lauraceae plants collected in this study and reported bioactivity.

Species	Activity	Reference
<i>Cinnamomum</i> Bl.		
<i>Cinnamomum kanehirai</i> Hay.	–	–
<i>Cinnamomum camphora</i> (L.) Ness et Eberm.	Anti-inflammatory, Antioxidant	Lee et al., 2006
<i>Cinnamomum philippinense</i> (Merr.) C. E. Chang	Thromboxane A2 receptor antagonist	Su et al., 1999
<i>Cinnamomum osmophloeum</i> Kanehira	Antioxidant, Antibacterial	Chang et al., 2001a
	Anti-mite	Chen et al., 2002
	Antitumor (Jurkat and U937 cells)	Fang et al., 2004
	Antifungal	Wang et al., 2005
	Anti-inflammatory	Chao et al., 2005
<i>Cinnamomum insularimontanum</i> Hay.	Antiviral	Lin et al., 2003
<i>Cinnamomum subavenium</i> Miq.	–	–
<i>Cinnamomum zeylanicum</i> Bl.	Antinociceptive	Atta & Alkofahi, 1998
	Antioxidant	Jayaprakasha et al., 2003
<i>Cinnamomum iners</i> Reinw. Ex Bl.	–	–
<i>Litsea</i> Lamk.		
<i>Litsea acuminata</i> (Bl.) Kurata	–	–
<i>Litsea rotundifolia</i> var. <i>oblongifolia</i> (Nees)	Antiplatelet aggregation, vasorelaxing	Yan et al., 2000
<i>Litsea kostermansii</i> Chang	–	–
<i>Litsea akoensis</i> Hay.	Antitumor (P-388, KB16, A549, and HT-29)	Chen et al., 1998
		Choi & Hwang, 2004
<i>Litsea cubeba</i> (Lour.) Persoon	Anti-inflammatory, antioxidant	Hwang et al., 2005
<i>Neolitsea</i> Merr.		
<i>Neolitsea parvigemma</i> (Hay.) Kanehira et Sasaki	Anti-inflammatory	Chen et al., 2005
<i>Neolitsea sericea</i> var. <i>aurata</i> (Hay.) Hatusima	Anti-mite	Furuno et al., 1994
<i>Neolitsea variabilima</i> (Hay.) Kaneh. et Sasaki	–	–
<i>Neolitsea konishii</i> (Hay.) Kanehira et Sasaki	Anti-inflammatory	Yu, 1994
<i>Nothaphoebe</i> Bl.		
<i>Nothaphoebe konishii</i> (Hay.) Hay.	–	–
<i>Machilus</i> Nees		
<i>Machilus zuihoensis</i> Hayata	Antitumor (HONE-1 and NUGC-3 cells)	Hou et al., 2003
	Antioxidant	Cheng et al., 2005
<i>Machilus thunbergii</i> Sieb. Et Zucc.	Anti-inflammatory	Kim & Ryu, 2003
<i>Machilus kusanoi</i> Hay		
<i>Lindera</i> Thunb		
<i>Lindera communis</i> Hemsl.	Antitumor (P-388, KB16, A549, and HT-29)	Tsai et al., 2002
<i>Lindera aggregate</i> (Sims) Kosterm.	Antioxidant	Mori et al., 2004
<i>Lindera megaphylla</i> Hemsl.	Vascular alpha 1-adrenoceptor antagonist	Yu et al., 1992
	Antitumor (HuH-7 and MS-G2)	Huang et al., 1998
<i>Beilschmiedia</i> Nees		
<i>Beilschmiedia erythrophloia</i> Hay.	–	–
<i>Cryptocarya</i> R. Brown		
<i>Cryptocarya concinna</i> Hance	–	–
<i>Phoebe</i> Nees		
<i>Phoebe formosana</i> (Matsum. et Hay.) Hay.	–	–

–, not found.

most environment-friendly strategy is not to utilize the entire tree, but to utilize its twigs and/or leaves. In our current study, the twigs and leaves from 27 tree species of Lauraceae grown in Taiwan were collected. Several bioassays, including antifungal activity, antioxidant activity, anti-inflammation activity, and cytotoxicity, were performed to evaluate potential bioactivity.

Materials and Methods

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), vitamin C, and lipopolysaccharide (LPS) were purchased from Sigma (St. Louis, MO, USA). All other

chemicals and solvents used in this study were of reagent or HPLC grade.

Plant and extract preparations

All of samples used in this study were collected from the Experimental Forest of National Taiwan University and Liukuei Education Center of Taiwan Forestry Research Institute in 2005 (Table 1). The samples were identified by Prof. Y.-H. Tseng (Department of Forestry, National Chung-Hsing University). Voucher specimens were deposited in the herbarium of the Department of Forestry, NCHU. The extracts were prepared by the following procedure. Fresh leaf and twig mixture (500 g) was extracted twice with 2.5 L of methanol at ambient temperature. The extracts were decanted, filtered under vacuum, concentrated in a rotary evaporator, and then lyophilized. The resulting powder extracts were employed for the current study.

Antifungal assay

The fungi used were *Trametes versicolor* (BCRC 35253) and *Laetiporus sulphureus* (BCRC 35305). *In vitro* antifungal assays were performed as in our previous study (Chang et al., 1999). Assays were carried out in triplicate, and data were averaged. Extracts (100 µg/mL) were added to sterilized potato dextrose agar (PDA). The testing Petri dishes were incubated in the dark at $26 \pm 2^\circ\text{C}$ and 70% relative humidity. When the mycelium of fungi reached the edges of the control Petri dishes, the antifungal indices were calculated. Each test was repeated three-times, and the data were averaged. The antifungal index was calculated as follows:

$$\text{Antifungal index (\%)} = \left(\frac{1 - \text{diameter}_{\text{experimental}}}{\text{diameter}_{\text{control}}} \right) \times 100.$$

Free radical scavenging activity

The scavenging activity for DPPH radicals by plant extracts from Lauraceae was measured according to the method as described previously (Chang et al., 2001b; Wang et al., 2002). Assays were performed in 300 µL reaction mixtures, containing 200 µL of 0.1 mM DPPH-ethanol solution, 90 µL of 50 mM Tris-HCl buffer (pH 7.4), and 10 µL of ethanol (as solvent blank) or test plant extracts and ascorbic acid were used as positive controls. After 30 min of incubation at room temperature, absorbance (540 nm) of the reaction mixtures was taken by ELISA reader (µQuant, Bio-Tek Instruments, Winooski, VT, USA). The inhibitory effect of DPPH was calculated according to the following formula:

$$\text{Inhibition(\%)} = \left(\frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \right) \times 100.$$

IC₅₀ represents the levels at which 50% of the radicals were scavenged by test samples.

Nitric oxide inhibition assay

Nitric oxide (NO) inhibition activities of Lauraceae extracts were conducted according to the method used previously (Wang et al., 2003). RAW 264.7 cells, a murine macrophage cell line, were obtained from ATCC (Rockville, MD, USA) and cultured at 37°C in Dulbecco's modified essential medium supplemented with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin in a 5% CO₂ incubator, as recommended by ATCC. RAW 264.7 cells grown in T75 culture flasks were harvested and seeded in 96-well plates at a density of 2×10^5 cells/well. Adhered cells were incubated for 24 h with (positive control) or without (negative control) 1 µg/mL LPS, in the absence or presence of test extracts. Nitrite (NO₂⁻) concentration, as a parameter of NO synthesis, in the culture supernatant of RAW 264.7 cells was measured by the Griess reaction (Schmidt & Kelm, 1996). Briefly, 100 µL cell culture supernatants were reacted with 100 µL of Griess reagent [1:1 mixture of 0.1% *N*-(1-naphthyl)ethylenediamine in H₂O and 1% sulfanilamide in 5% phosphoric acid] in a 96-well plate, and absorbance was recorded using an ELISA reader (µQuant) at 540 nm. Results were expressed as a percentage of inhibition relative to the control (cell treated with LPS alone). In parallel to the Griess assays, RAW 264.7 cells treated with or without the extracts were tested for cell viability using the MTT (4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) based colorimetric assay (Scudiero et al., 1988).

Tumor cell growth inhibition assay

Cytotoxicity was performed using a MTT assay (Alley et al., 1988; Song et al., 1994; Chang et al., 2000). Tumor cells [human tumor cells including HUVEC (human umbilical vein endothelial cell), MCF-7 (breast adenocarcinoma), and HL-60 (human leukemia)] (1×10^5 cells/mL) were seeded into a 96-well plate in triplicate and preincubated for 12 h in order to perform cell attachment. Then, 100 µL fresh medium containing various concentrations (500, 250, 100, 50, and 25 µg/mL of methanol extracts) of test compound were added into the 96-well plate. The cells were incubated with each compound at 37°C for 24 h under humidified air containing 5% CO₂. Cell survival was evaluated by adding 100 µL tetrazolium salt solution (1 mg MTT/mL in PBS). After 4 h of incubation at 37°C, 100 µL DMSO was added to dissolve the precipitates of reduced MTT. Microplates were then shaken for 15 min, and the absorbance was determined at 570 nm in a multiwell scanning spectrophotometer.

Results and Discussion

Antifungal activity of methanol extracts from Lauraceae

To evaluate the antifungal activities of extracts from Lauraceae against fungi, we selected two representative fungi, *T. versicolor* (white-rot fungus) and *L. sulphureus* (brown-rot fungus) as testing strains. According to the results obtained from antifungal assays, the antifungal indices were lower than 10.0% against both fungi at the dosage of 100 µg/mL (data not show), indicating the extracts of Lauraceae examined in this study did not show a significant antifungal activity. Wang and his co-workers (2005) have demonstrated that the essential oils of *C. osmophloeum* possessed strong antifungal activity. Surprisingly, the methanol extracts of *C. osmophloeum* did not show the expected antifungal activity in this study. It might be due to the low amount of active component (cinnamaldehyde) in the methanol extracts of *C. osmophloeum* (Wang et al., 2005). Overall, the antifungal performance of Lauraceae tree species studied herein was not considerable.

Radical scavenging activities of methanol extracts from Lauraceae

The extracts from Lauraceae tree species were tested for their capacity to scavenge free radicals of DPPH, which has been used to evaluate the antioxidant activity of natural products from plants globally (Wang et al., 2002). The results of DPPH scavenging activities are shown in Table 2. Most extracts from Lauraceae revealed good scavenging activities for DPPH radicals. The EC₅₀ of four species, including *C. subarenium* (EC₅₀ = 6.12 µg/mL), *L. acuminata* (EC₅₀ = 6.85 µg/mL), *N. parvigemma* (EC₅₀ = 5.73 µg/mL), and *N. variabilima* (EC₅₀ = 7.41 µg/mL), were lower than 10 µg/mL. In comparison with well-known antioxidants, ascorbic acid (EC₅₀ = 1.5 µg/mL) and quercetin (EC₅₀ = 2.3 µg/mL), the crude extracts of the trees mentioned above exhibited good antioxidant activity. Reactive oxygen species (ROS) are essential for life for they are involved in cell physiology. However, over production of ROS is suggested to be strongly associated with the aging process and certain degenerative diseases including various cancers, cognitive dysfunctions, and coronary heart disease (Finkle & Holbrook, 2000). Thus, it is important to discover effective antioxidants from natural sources, especially from plant species, to reduce ROS activities. On the basis of the study using *in vitro* DPPH radical scavenging assay, we suggest that Lauraceae plants, such as *C. subarenium*, *L. acuminata*, *N. parvigemma*, and *N. variabilima*, are potential candidates to serve as supplements for human health care.

Table 2. DPPH free radical scavenging activities of extracts from 27 Lauraceae tree species.

Species	EC ₅₀ (µg/mL)
Cinnamomum	
<i>Cinnamomum kanehirai</i>	>100
<i>Cinnamomum camphora</i>	>100
<i>Cinnamomum philippinense</i>	10.06 ± 0.74
<i>Cinnamomum osmophloeum</i>	11.84 ± 1.36
<i>Cinnamomum insularimontanum</i>	27.99 ± 0.01
<i>Cinnamomum subavenium</i>	6.12 ± 0.08
<i>Cinnamomum zeylanicum</i>	13.95 ± 1.32
<i>Cinnamomum iners</i>	23.27 ± 1.05
Litsea	
<i>Litsea acuminata</i>	6.85 ± 0.13
<i>Litsea rotundifolia</i> var. <i>oblongifolia</i>	14.04 ± 0.20
<i>Litsea kostermansii</i>	12.80 ± 0.85
<i>Litsea akoensis</i>	22.53 ± 4.18
<i>Litsea cubeba</i>	11.39 ± 0.38
Neolitsea	
<i>Neolitsea parvigemma</i>	5.73 ± 0.37
<i>Neolitsea sericea</i> var. <i>aurata</i>	30.57 ± 4.66
<i>Neolitsea variabilima</i>	7.41 ± 0.13
<i>Neolitsea konishii</i>	31 ± 2.71
Nothaphoebe	
<i>Nothaphoebe konishii</i>	18.23 ± 2.53
Machilus	
<i>Machilus zuihoensis</i>	11.43 ± 1.13
<i>Machilus thunbergii</i>	>100
<i>Machilus kusanoi</i>	18.78 ± 0.63
Lindera	
<i>Lindera communis</i>	11.88 ± 1.35
<i>Lindera aggregate</i>	11.28 ± 0.22
<i>Lindera megaphylla</i>	37.47 ± 0.06
Beilschmiedia	
<i>Beilschmiedia erythrophloia</i>	13.51 ± 0.59
Cryptocarya	
<i>Cryptocarya concinna</i>	12.7 ± 0.68
Phoebe	
<i>Phoebe formosana</i>	86.5 ± 1.16
Ascorbic acid	1.5 ± 0.01
Quercetin	2.3 ± 0.01

EC₅₀: 50% DPPH free radical scavenging concentration.

Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells

Activation of macrophages plays a critical role in the inflammatory process by releasing a variety of inflammatory mediators (Zhuang et al., 1998), such as NO, which is a critical signaling molecule produced at inflammatory sites by inducible nitric oxide synthase (iNOS), which is often expressed in response to LPS and a variety of proinflammatory cytokines (MacMicking et al., 1997). In this study, the effects of methanol extracts from Lauraceae on NO synthesis in RAW 264.7 macrophages were investigated. As shown in Figure 1, Lauraceae methanol extracts exhibited significant inhibition of nitrite production.

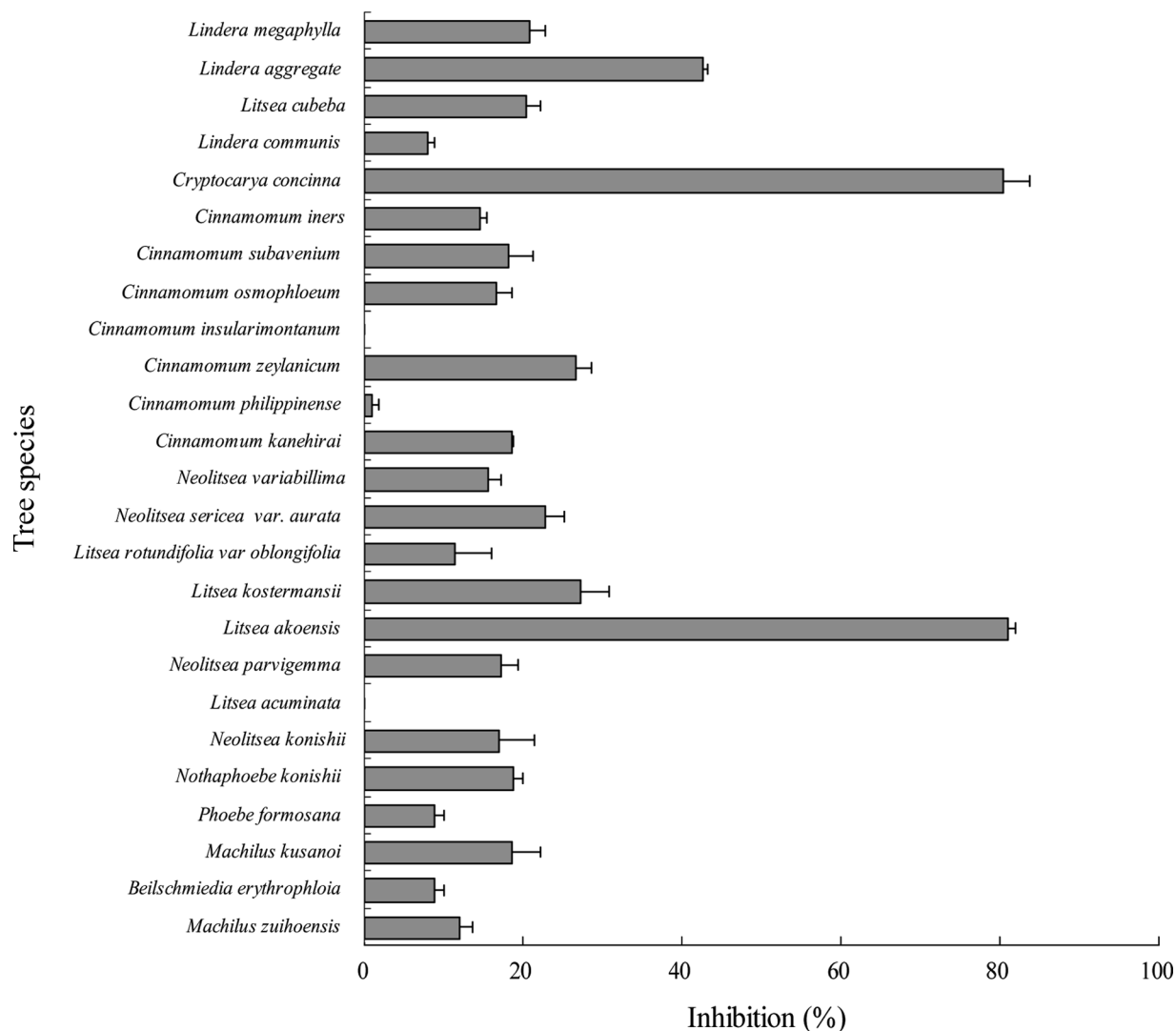


Figure 1. Anti-inflammatory activity of 25 tree species extracts by using NO free radical inhibition assay. (dose = 25 $\mu\text{g}/\text{mL}$).

Among the test extracts, *Litsea akoensis* and *Cryptocarya concinna* extracts exhibited the most significant inhibitory activity; 81.07% and 80.37% of NO production was inhibited at the dose of 25 $\mu\text{g}/\text{mL}$, respectively.

Tumor cell growth inhibition assay

Cytotoxicity of Lauraceae extracts was evaluated by a MTT assay, which measures the relative metabolic rate activity of the cells (Alley et al., 1988; Song et al., 1994). The cytotoxicity of the methanol extracts from Lauraceae was tested against HUVEC, MCF-7, and HL-60 cell lines in this study. As shown in Table 3, *Lindera aggregate* ($\text{IC}_{50} = 43.15 \mu\text{g}/\text{mL}$), *Cryptocarya concinna* ($\text{IC}_{50} = 49.36 \mu\text{g}/\text{mL}$), and *Phoebe formosana* ($\text{IC}_{50} = 42.87 \mu\text{g}/\text{mL}$) showed significant cytotoxicity for HUVEC and HL-60, respectively. However, the methanol extracts did not display any cytotoxicity against the MCF-7 cancer cell line. On the basis of the

results obtained, effective antitumor active compounds from the methanol extracts of *Lindera aggregate*, *Cryptocarya concinna*, and *Phoebe formosana* can be obtained when further separation and purification are carried out in the near future.

Conclusions

The extracts from 27 woody plants of Lauraceae grown in Taiwan were assayed to explore their bioactivities. The results indicated that a number of extracts present significant activities, such as antioxidant, anti-inflammation, antitumor activities. This study provides valuable and useful information and indications for further exploring the potential nutraceutical and pharmaceutical applications of the Lauraceae tree species. Further investigations will be conducted by our research team.

Table 3. Cytotoxicity activity of 15 tree species extracts against HUVEC, HL-60, and MCF-7 cells.

Species	EC ₅₀ (µg/mL)		
	HUVEC	HL-60	MCF-7
Cinnamomum			
<i>Cinnamomum osmophloeum</i>	>100	>100	>100
<i>Cinnamomum subavenium</i>	>100	>100	>100
<i>Cinnamomum iners</i>	>100	95.08 ± 1.08	>100
Litsea			
<i>Litsea acuminata</i>	>100	>100	>100
<i>Litsea rotundifolia</i> var <i>oblongifolia</i>	>100	>100	>100
<i>Litsea cubeba</i>	>100	>100	>100
Neolitsea			
<i>Neolitsea variabilima</i>	>100	>100	>100
<i>Neolitsea konishii</i>	>100	>100	>100
Machilus			
<i>Machilus zuihoensis</i>	>100	>100	>100
<i>Machilus kusanoi</i>	>100	>100	>100
Lindera			
<i>Lindera aggregate</i>	43.15 ± 1.57	>100	>100
<i>Lindera megaphylla</i>	>100	>100	>100
Beilschmiedia			
<i>Beilschmiedia erythrophloia</i>	>100	>100	>100
Cryptocarya			
<i>Cryptocarya concinna</i>	49.36 ± 5.62	>100	>100
Phoebe			
<i>Phoebe formosana</i>	>100	42.87 ± 2.24	>100

EC₅₀; inhibition 50% cell survived concentration.

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