



Evaluation of Anti-Asthmatic Activity of Essential Oils from the Lauraceae Family in Lipopolysaccharide (LPS)-Stimulated NCI-H292 Cells

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ABSTRACT

The Lauraceae family has commercial uses, such as in the food, pharmaceutical, and perfume industries. This study was conducted to investigate anti-asthmatic activity of essential oils from the seven species in the Lauraceae family. The essential oils were extracted from the leaves of seven species, and the chemical composition was investigated by gas chromatography-mass spectrometry. The major constituents of essential oils differed depending on the species, even if they belonged to the same family. The main constituents were camphor (89.09%) in *Cinnamomum camphora*, linalool (26.91%) in *Cinnamomum cassia*, 1,8-cineole (23.90%) in *Cinnamomum japonicum*, d-limonene (10.27%) and β -eudesmol (10.03%) in *Lindera obtusiloba*, δ -cadinene (13.85%) and α -phellandrene (11.57%) in *Machilus japonica*, *cis*-,*trans*- β -ocimene (13.80% and 12.06%) and elemol (11.46%) in *Neolitsea aciculata*, and *cis*- β -ocimene (37.94%) and sabinene (24.91%) in *Neolitsea sericea*. The anti-asthmatic activity of essential oils was investigated using the lipopolysaccharide-induced NCI-H292 cells. The relative expression levels of the pro-inflammatory cytokines [interleukin (IL)-1 β and IL-6] and mucus gene (MUC5AC and MUC5B) were significantly reduced by essential oils from seven species in the Lauraceae family. Among the seven essential oils, the essential oil from *L. obtusiloba* had the most superior anti-asthmatic activity. These results suggest that the essential oil of *L. obtusiloba* leaves could be used as an agent to suppress mucus hypersecretion.

Keywords: Lauraceae, *Lindera obtusiloba*, anti-asthmatic activity, essential oil, mucin, NCI-H292

1. INTRODUCTION

The Lauraceae family consists of aromatic trees that are predominantly found in Asia as well as North and South America. There are approximately 2,500–3,500 species distributed throughout the world (Silva *et al.*, 2009). Many species of the Lauraceae family have commercial uses, such as in the food, pharmaceutical, and

perfume industries (Chau *et al.*, 2020). Previous studies reported biological activities, such as antioxidant, anti-fungal, antibacterial, anti-inflammatory, anti-tyrosinase, antileishmanial, anticholinesterase, anti-melanogenic activity, etc., for the essential oils (EOs) isolated from the Lauraceae family (Damasceno *et al.*, 2019).

Natural products are increasingly popular with the growing interest in human health (Min *et al.*, 2017). The

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EO is one of the secondary metabolites derived from aromatic plants, and it consist of volatile compounds (Ham *et al.*, 2020). The EOs can relieve certain symptoms including anxiety, pain, insomnia, and bacterial infections (Ali *et al.*, 2015; Kim *et al.*, 2020). It is known to have bioactivities such as anti-fungal, anti-insect, anti-bacterial, and anti-inflammatory, and antioxidant activities (Jang *et al.*, 2012; Lee *et al.*, 2014; Pujiarti and Kasmudjo, 2016; Yang *et al.*, 2019; Yang *et al.*, 2022). EOs obtained from some species have been traditionally used to treat various respiratory ailments, which are associated with mucus hypersecretion and accumulation in the airways, such as asthma and chronic obstructive pulmonary disease (Asif *et al.*, 2020; Thai *et al.*, 2008). However, there are no reports demonstrating an inhibitory effect of the EO of the Lauraceae family on mucus secretion in the airway.

Asthma is a common chronic disease that affected an estimated 334 million people worldwide, it causes approximately 25 million deaths annually (Bonser and Erle, 2017). Asthma causes symptoms such as wheezing, coughing, and chest tightness due to airflow obstruction caused by inflamed airway smooth muscle and impaired mucosal (Fanta, 2009). Airway mucus hypersecretion is a feature of many asthmatics and indicates poor asthma control (Rogers, 2004). Excessive mucus production not only obstructs airways but also contributes to airway hyperresponsiveness (Evans *et al.*, 2009). Therefore, various studies on anti-hypersecretory agents for regulating airway mucus hypersecretion in asthma are currently being conducted (Ha and Rogers, 2016).

Inflammatory mediators [interleukin (IL)-1 β and IL-6] commonly upregulated during respiratory inflammatory responses increase MUC5AC mRNA in lung epithelial cells and transcriptionally upregulate MUC5AC gene expression (Chen *et al.*, 2014). This interaction suggested that pro-inflammatory cytokines could induce mucin gene expression by cytokine receptors with the regulatory regions of the mucin gene (Wang *et al.*, 2007).

Mucins are high-molecular weight glycoproteins and are the predominant constituents that impart the viscoelastic and gel-forming properties to mucus (Williams *et al.*, 2006). The 20 mucin genes that are known to be expressed in the lungs include MUC1, -2, -4, -5AC, -5B as well as others (Rose and Voynow, 2006). Of these, MUC5AC and MUC5B are considered the predominant mucins in airway mucus, because they are the only ones consistently found to be expressed at the protein level (Thornton *et al.*, 2000). This study was determined whether EOs can suppress mucin gene expression in NCI-H292 epithelial cells *in vitro*.

In this study, the EOs from seven species [*Cinnamomum camphora* (L.) J. Presl, *Cinnamomum cassia* (L.) J. Presl, *Cinnamomum japonicum* Siebold, *Lindera obtusiloba* Blume, *Machilus japonica* Siebold & Zucc., *Neolitsea aciculata* (Blume) Koidz., and *Neolitsea sericea* (Blume) Koidz.] in Lauraceae were evaluated the inhibitory effects on the pro-inflammatory cytokines and mucin genes. In addition, the chemical composition of EOs were investigated using gas chromatography-mass spectrometry.

2. MATERIALS and METHODS

2.1. Plant materials and extraction of the essential oils (EOs)

The leaves of *C. camphora* (L.) J. Presl, *C. cassia* (L.) J. Presl, *C. japonicum* Siebold, *N. aciculata* (Blume) Koidz., and *N. sericea* (Blume) Koidz. were collected from Jeju Island. The leaves of *L. obtusiloba* Blume and *M. japonica* Siebold & Zucc. were obtained from Yeongju and Wando, respectively. Information on collection sites is summarized in Table 1.

The Lauraceae family EOs were extracted from the leaves of *C. camphora*, *C. cassia*, *C. japonicum*, *L. obtusiloba*, *M. japonica*, *N. aciculata*, and *N. sericea* by hydro-distillation method. A 10 L round-bottom flask

Table 1. Voucher information

Scientific name	Latitude	Longitude
<i>Cinnamomum camphora</i> (L.) J. Presl	33 ° 18' 43.43"N	126 ° 33' 50.19"E
<i>Cinnamomum cassia</i> (L.) J. Presl	33 ° 17' 54.05"N	126 ° 37' 08.04"E
<i>Cinnamomum japonicum</i> Siebold	33 ° 20' 34.51"N	126 ° 39' 36.36"E
<i>Lindera obtusiloba</i> Blume	35 ° 12' 13.01"N	128 ° 10' 05.01"E
<i>Machilus japonica</i> Siebold & Zucc.	33 ° 17' 14.18"N	126 ° 16' 12.86"E
<i>Neolitsea aciculata</i> (Blume) Koidz.	33 ° 20' 05.62"N	126 ° 39' 32.73"E
<i>Neolitsea sericea</i> (Blume) Koidz.	33 ° 20' 34.51"N	126 ° 39' 36.36"E

containing 1.0 kg based fresh weight of the leaves was poured 6 L of distilled water and placed on a digital heating mantle (MS-DM608 heating mantle, MTOPS®, Misung Scientific, Yangju, Korea), then connected to the Clevenger-type apparatus. To prevent the volatile components of the EOs from evaporating, a refrigerant was circulated in the condenser to -5°C and the mixtures were heated to $104 \pm 2^{\circ}\text{C}$ for 6 h. The EOs were stored at 4°C until used. The EO yield was determined by the following Equation (1).

$$\text{Essential oil yield (\%)} = \frac{\text{Mass of essential oil (g)}}{\text{Mass of dried sample (g)}} \times 100 \quad (1)$$

2.2. Chemical composition analysis of the essential oils (EOs)

The volatile components of the EOs were analyzed using Trace 1300/ISQ-LT gas chromatography-mass spectrometry (GC-MS, Thermo Fisher Scientific, Waltham, MA, USA) fitted with a Tr-5MS column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$; Thermo Fisher Scientific). The injector temperature was set at 250°C , and helium (He, 1.0 mL/in, 25 psi) was used as the carrier gas. The oven temperature was programmed to 40°C for 3 min and then increased to 200°C at a temperature increase rate of 3°C

/min. The temperature was then raised at $15^{\circ}\text{C}/\text{min}$ to 340°C and maintained for 10 min. Flame ionization detector (FID) signals and mass spectra of the detected compounds were recorded. The FID was set at 280°C and the flow rate of the helium make-up gas was 40.0 mL/min. The temperature of the mass spectrometer interface and the ion source were 280°C and 250°C , respectively. The mass spectra were recorded at a rate of 0.2 scans/s in the EI-scan range of 35–360 m/z. *n*-alkanes (C_7 – C_{29} , Sigma-Aldrich, St. Louis, MO, USA) were used to calculate the retention indices of the components. Compared with the NIST 11 (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library, the compounds with the highest match values were listed as the compound names.

2.3. Cell culture

The human bronchial epithelial cell line NCI-H292 (KCLB No. 21848) was obtained from the Korea Cell Line Bank (Seoul, Korea). NCI-H292 cells were cultured in RPMI-1640 (Welgene, Gyeongsan, Korea) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 1% penicillin-streptomycin (Gibco), and $0.4 \mu\text{L}/\text{mL}$ plasmocin (InvivoGen, San Diego, CA, USA) at 37°C in a 5% CO_2 incubator (Panasonic, Osaka, Japan).

2.4. Cell cytotoxicity of the essential oils (EOs)

Cell cytotoxicity of seven EOs on NCI-H292 cells was examined using Cell Counting Kit-8 (CCK-8, DoGenBio, Seoul, Korea) according to the previous study (Yang *et al.*, 2021a). The EOs were prepared in dimethyl sulfoxide (DMSO, Sigma-Aldrich). The cells were seeded into 96-well plates at a density of 8,000 cells/well and incubated at 37°C in a 5% CO₂ incubator for 24 h. Thereafter, the culture medium was removed and washed with Dulbecco's phosphate-buffered saline (DPBS, Gibco). The cells were treated with concentration at 10⁻⁷-10⁻⁴% of the EOs and cultured under the same conditions described above. After treatment, the cells were washed twice with DPBS and treated with 100 μL of 10% CCK-8 reagent. The plates were then incubated at 37°C for 1 h. Then, the optical density value of the plates was measured using a microplate reader (Epoch, BioTek Instruments, Winooski, VT, USA) at 450 nm. The cell viability was calculated using the following Equation (2).

$$\text{Cell viability (\%)} = \frac{(A_t - A_b)}{(A_c - A_b)} \times 100 \quad (2)$$

Where, A_t is the absorbance of treated cells, A_b is the absorbance of the blank, and A_c is the absorbance of the control.

2.5. mRNA quantitative analysis by real-time polymerase chain reaction (RT-PCR)

Total RNA was isolated from NCI-H292 cells as done in a previous study (Yang *et al.*, 2021b). NCI-H292 cells were cultured in 6-well plates (1 × 10⁶ cells/well) for 24 h. After washing the cells with DPBS, the cells were stimulated with 1 μg/mL lipopolysaccharide (LPS, Sigma-Aldrich) for 1 h and then washed twice with

DPBS. The cells were treated with 5 μg/mL budesonide and the seven EOs diluted at an appropriate concentration for 24 h. The cells were washed twice with DPBS and RNA was isolated using TRIzol reagent (Invitrogen, Waltham, MA, USA) and quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific) at 260 nm. Each cDNA samples were synthesized from the RNA and quantitative analysis was carried out using a CFX96 real-time PCR (Bio-Rad Laboratories, Hercules, CA, USA). The reaction volume was 10 μL containing 5 μL SYBR® reaction mix (1X final, Bio-Rad Laboratories), 1 μL each primer (3 pmol), 1 μL DNA template, and 2 μL distilled water. Thermal cycling was performed with an initial denaturation at 95°C for 1 min followed by 40 cycles of denaturation at 95°C for 10 s, and an annealing step at 60°C for 30 s. The oligonucleotide primers used for PCR amplification are listed in Table 2.

2.6. Statistical analysis

Each experiment was performed at least three times. Statistical analysis was done using a repeated one-way analysis of variance with Statistical Package for the Social Sciences software (SPSS ver. 17.0, SPSS, Chicago, IL, USA). Values are expressed as the mean ± SD. p values less than 0.05 were considered statistically significant.

3. RESULTS and DISCUSSION

3.1. Yields and chemical composition of essential oils (EOs)

EOs from seven species belonging to the Lauraceae family were obtained by hydro-distillation. The yields were 1.45 ± 0.64% in *C. camphora* leaves, 0.58 ± 0.0% *C. cassia* leaves, 0.57 ± 0.06% in *C. japonicum* leaves, 0.25 ± 0.05% in *L. obtusiloba* leaves, 0.33 ± 0.06% in *M. japonica* leaves, 0.28 ± 0.09% in *N. aciculata* leaves,

Table 2. Oligonucleotide sequences for quantitative RT-PCR

Gene	Primer sequence (5'-3')
<i>MUC5AC</i>	F: 5'-TGA TCA TCC AGC AGC AGG GCT-3'
	R: 5'-CCG AGC TCA GAG GAC ATA TGG G-3'
<i>MUC5B</i>	F: 5'-CAC ATC CAC CCT TCC AAC-3'
	R: 5'-GGC TCA TTG TCG TCT CTG-3'
<i>Rig/SI5</i>	F: 5'-TTC CGC AAG TTC ACC TAC C-3'
	R: 5'-CGG GCC GGC CAT GCT TTA CG-3'
<i>IL-1β</i>	F: 5'-CAC ATG GGA TAA CGA GGC TT-3'
	R: 5'-TGG AGA ACA CCA CTT GTT GC-3'
<i>IL-6</i>	F: 5'-GCA CTG GCA GAA AAC AAC CT-3'
	R: 5'-TCA AAC TCC AAA AGA CCA GTG A-3'
<i>GAPDH</i>	F: 5'-TGC ACC ACC AAC TGC TTA GC-3'
	R: 5'-GGC ATG GAC TGT GGT CAT GAG-3'

RT-PCR: real-time polymerase chain reaction, IL: interleukin, F: forward, R: reverse, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

and $0.38 \pm 0.16\%$ *N. sericea* leaves. The highest yield was observed in *C. camphora* and the lowest in *L. obtusiloba*.

The chemical composition of the EOs is summarized in Table 3 only for components with 3% or more. The chemical composition of EOs depends on the plant species, even if they belong to the same family (Ahn *et al.*, 2018). The major component of EO from *C. camphora* was camphor (89.09%). Linalool (26.91%), (*E*)-citral (10.69%), and (*Z*)-citral (8.46%) were identified as the major components of EO from *C. cassia*. The major components of EO from *C. japonicum* were 1,8-cineole (23.90%), α -phellandrene (11.75%), and β -myrcene (9.04%). D-limonene (10.27%) and β -eudesmol (10.03%) were major components in EO from *L. obtusiloba*. The major components of EO from *M. japonica* were δ -cadinene (13.85%), α -phellandrene (11.57%), and τ -

muurolol (9.52%). *cis*-, *trans*- β -Ocimene (13.80% and 12.06%), elemol (11.46%), and β -elemene (10.94%) were identified as the major components of *N. aciculata* oil. The major component of EO from *N. sericea* were *cis*- β -ocimene (37.94%) and sabinene (24.91%). In case of *C. camphora* oil, *M. japonica* oil, and *N. sericea* oil, the results in the present study were similar to the results of previous studies (Pragadheesh *et al.*, 2013; Van Khien *et al.*, 2009; Yoon *et al.*, 2010). However, the results of *C. cassia* oil, *C. japonicum* oil, *L. obtusiloba* oil, and *N. aciculata* oil were different from the result of previous study (Chahbi *et al.*, 2020; Hwang *et al.*, 2013; Kim *et al.*, 2011; Zhang *et al.*, 2018). The major components of *C. cassia* oil, *C. japonicum* oil, *L. obtusiloba* oil, and *N. aciculata* oil in previous studies were found as minor component in this study. There was a difference in chemical composition of EOs according to the growing environment of plants (Chrysargyris *et al.*, 2022). Especially, chemical compositions are related to exogenous factors such as light, geographical location, precipitation, harvesting time, and soil conditions (Şanlı and Karadoğan, 2017; Zouari *et al.*, 2012). When plants are exposed to other exogenous factors for a long time, it could undergo genetic modification, which causes a change in biosynthetic pathways, leading to the qualitative/quantitative variation of secondary metabolites (Barra, 2009).

3.2. Cell cytotoxicity of essential oils (EOs)

Natural products can interact with DNA and cause changes in its structure of function, so the appropriate concentration of EO should be determined by assessing the cell cytotoxicity (Ahn *et al.*, 2021). To determine the cell toxicity of the EOs, NCI-H292 cell viability was measured at various concentrations ($10^{-7}\%$ – $10^{-4}\%$) of the seven EOs (Fig. 1). The cell viability was more than 80% at concentrations of $10^{-7}\%$ – $10^{-4}\%$, and no cytotoxicity was observed at concentrations lower than $10^{-4}\%$. Based on the cytotoxicity results, the inhibitory effect

Table 3. Chemical compositions of essential oils derived from the Lauraceae family leaves

RI ¹⁾	Compound name	<i>Cinnamomum camphora</i>	<i>Cinnamomum cassia</i>	<i>Cinnamomum japonicum</i>	<i>Lindera obtusiloba</i>	<i>Machilus japonica</i>	<i>Neolitsea aciculata</i>	<i>Neolitsea sericea</i>
926	α -Pinene	0.40	2.22	4.06	5.73	4.11	0.07	2.95
938	Camphene	0.51	0.58	0.70	5.14	3.67	tr ²⁾	0.03
966	Sabinene	tr	-	2.65	-	0.04	0.10	24.91
971	β -Pinene	0.14	1.00	1.61	4.79	1.02	0.04	4.53
980	β -Myrcene	0.18	-	9.04	1.05	1.03	0.21	2.22
1001	α -Phellandrene	0.04	-	11.75	0.72	11.57	0.21	0.11
1030	<i>trans</i> - β -Ocimene	tr	0.30	1.57	1.56	2.57	12.06	1.12
1038	<i>cis</i> - β -Ocimene	tr	-	1.16	-	3.58	13.80	37.94
1039	D-Limonene	0.33	0.51	1.96	10.27	0.65	0.03	1.44
1042	1,8-Cineole	0.03	3.17	23.90	0.10	0.10	0.23	0.77
1105	Linalool	0.01	26.91	6.28	-	0.15	0.93	0.43
1153	Camphor	89.09	-	0.54	2.67	tr	0.01	0.01
1179	Borneol	0.51	0.33	0.02	3.52	0.03	tr	tr
1189	Terpinen-4-ol	0.18	0.41	2.65	0.15	0.02	0.03	6.13
1195	α -Terpineol	0.50	1.04	5.02	1.18	0.13	0.03	0.45
1244	(<i>Z</i>)-Citral	tr	8.46	0.22	-	tr	tr	tr
1256	Geraniol	tr	6.12	0.26	-	tr	tr	0.01
1273	(<i>E</i>)-Citral	tr	10.69	tr	-	tr	tr	tr
1286	Bornyl acetate	tr	1.04	0.07	5.69	3.58	tr	0.03
1380	Geranyl acetate	tr	7.62	0.06	-	0.06	0.08	0.40
1397	β -Elemene	0.09	-	0.26	1.72	1.22	10.94	0.84
1433	β -Caryophyllene	0.70	0.75	1.57	3.43	1.28	7.38	1.12
1449	Cinnamyl acetate	-	5.27	-	-	-	-	-
1495	Germacrene D	0.01	-	0.34	0.38	0.09	9.45	0.58
1499	α -Zingiberene	-	3.51	-	-	-	-	-
1508	α -Muurolene	tr	-	0.12	-	4.18	0.61	0.06
1510	γ -Elemene	1.08	-	0.20	2.65	1.34	6.74	2.36
1532	β -Cadinene	0.04	0.67	0.54	1.93	13.85	0.03	0.30
1544	Elemol	0.11	-	1.37	-	tr	11.46	0.05
1566	Hedycaryol	-	-	-	6.79	-	-	-
1619	Viridiflorol	0.33	-	0.69	0.43	3.46	0.39	0.74
1653	β -Cadinol	0.14	-	0.26	-	4.58	0.42	0.14
1665	τ -Cadinol	0.15	-	0.38	2.04	7.23	1.06	0.18
1668	τ -Muurolol	0.46	-	1.80	1.82	9.52	6.18	0.49
1680	α -Cadinol	0.18	-	0.52	4.27	7.23	1.14	0.22
1683	β -Eudesmol	0.13	-	0.64	10.03	0.24	2.96	0.03
	Unknown compound	0.92	8.98	2.50	9.04	4.51	1.43	0.13

¹⁾ Retention indice (RI): Kovats retention index was experimentally determined using a Tr-5MS column.²⁾ tr (=trace) < 0.01%.

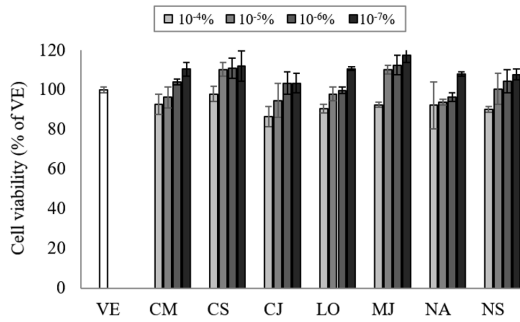


Fig. 1. Cell toxicity of seven EOs on NCI-H292 cells. Values are expressed as the mean ± SD. VE: vehicle, CM: *Cinnamomum camphora*, CS: *Cinnamomum cassia*, CJ: *Cinnamomum japonicum*, LO: *Lindera obtusiloba*, MJ: *Machilus japonica*, NA: *Neolitsea aciculata*, NS: *Neolitsea sericea*, EOs: essential oils.

on gene expression was performed at a concentration of 10⁻⁷%.

3.3. Effects of essential oils (EOs) on the gene expression of pro-inflammatory cytokine

Several cytokines induce MUC5AC and MUC5B gene expression and mucus hypersecretion (Kim, 2007). The inflammatory mediators such as IL-1β and IL-6 are responsible for mucin hypersecretion in inflammatory diseases of the lung and gastrointestinal tract (Van Seuning et al., 2001). The EOs from the seven species significantly inhibited the expression of the pro-inflammatory cytokines in LPS-stimulated NCI-H292 cells (Fig. 2).

The IL-1β gene expression of LPS-stimulated NCI-H292 cells [negative control (NC)] was approximately 1.4-fold higher than that of the untreated cells [vehicle (VE); Fig. 2(a)]. A positive control (PC) group treated with budesonide down-regulated gene expression by 6.7% compared to NC. The treatment of EOs on LPS-stimulated NCI-H292 cells reduced IL-1β gene expression by 19.3%–47.4% compared to NC. Among seven

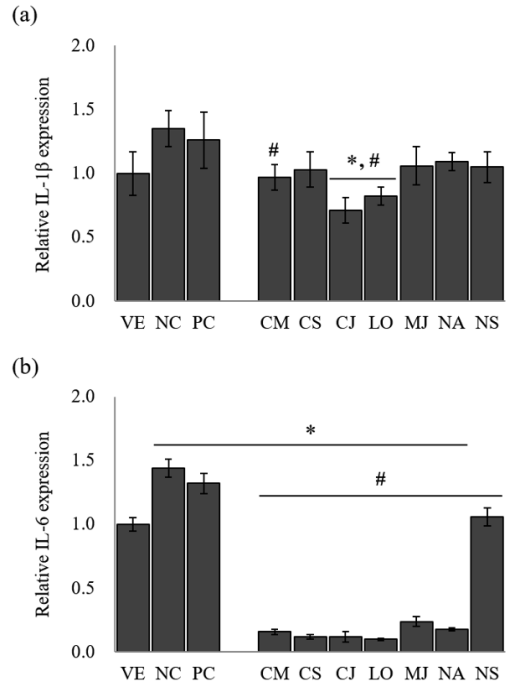


Fig. 2. Effects of seven EOs on LPS-induced pro-inflammatory cytokine production: (a) IL-1β, (b) IL-6 gene expressions. Values are expressed as the mean ± SD. * *p* < 0.05 compared with VE; # *p* < 0.05 compared with NC. IL: interleukin, VE: vehicle, NC: negative control (1 μg/mL lipopolysaccharide), PC: positive control (5 μg/mL budesonide), CM: *Cinnamomum camphora*, CS: *Cinnamomum cassia*, CJ: *Cinnamomum japonicum*, LO: *Lindera obtusiloba*, MJ: *Machilus japonica*, NA: *Neolitsea aciculata*, NS: *Neolitsea sericea*, EOs: essential oils, LPS: lipopolysaccharide.

species, IL-1β gene expression showed the highest inhibitory effect in *C. japonicum* and *L. obtusiloba*, with 47.4% and 39.3% reduction, respectively.

LPS-stimulated NCI-H292 cells significantly increased the IL-6 gene expression level [Fig. 2(b)]. However, treatment with budesonide suppressed the IL-6 gene expression compared with NC by 8.3%. Similarly, treatment with EOs significantly reduced the IL-6 gene expression by 26.4%–93.1%. The EOs of *L. obtusiloba* (about 93.1% reduction), *C. japonicum* (about 91.7%

reduction), and *C. cassia* (about 91.7% reduction) effectively inhibited the IL-6 gene expression from the LPS-stimulated NCI-H292 cells.

IL-1 β is a prototypical inflammatory cytokine implicated in many inflammatory diseases and is a useful probe to evaluate mucin secretion. In particular, it is known to stimulate mucin gene MUC5AC expression (Gray *et al.*, 2004). IL-6 has also been reported to induce MUC5AC gene expression (Hashimoto *et al.*, 2004). These results suggest that EOs of *L. obtusiloba* and *C. japonicum* have a possibility for the suppression of hypersecretion of airway mucus in asthma. However, further studies will be performed to clarify the effect of each component of EOs on the asthma animal model.

3.4. Effects of essential oils (EOs) on the mucin gene expression

MUC5AC and MUC5B, the predominant gel-forming mucins, have been informed to be expressed in human airway epithelium, and are known as the predominant gel-forming mucins in normal or pathologic airway secretions (Henke *et al.*, 2003; Okuda *et al.*, 2019). To identify the inhibitory effect of mucus hypersecretion, the expression of the MUC5AC and MUC5B genes was evaluated in response to treatment with the seven EOs (Fig. 3).

The NC group showed a remarkable increase of 3.0-fold in MUC5AC gene expression compared to LPS-stimulated NCI-H292 cells. Budesonide inhibited the MUC5AC expression by 57.9% compared to NC. The treatment of EOs reduced MUC5AC gene expression by 1.3%–52.2% compared to NC. Among the EOs, MUC5AC gene expression showed the highest inhibitory effect by *L. obtusiloba* and *N. sericea*, with 52.2% and 50.8% reduction, respectively.

In the case of MUC5B, another secretory mucinous, the EO inhibited the gene expression of MUC5B, effectively. MUC5B expression was increased by 1.2-fold in

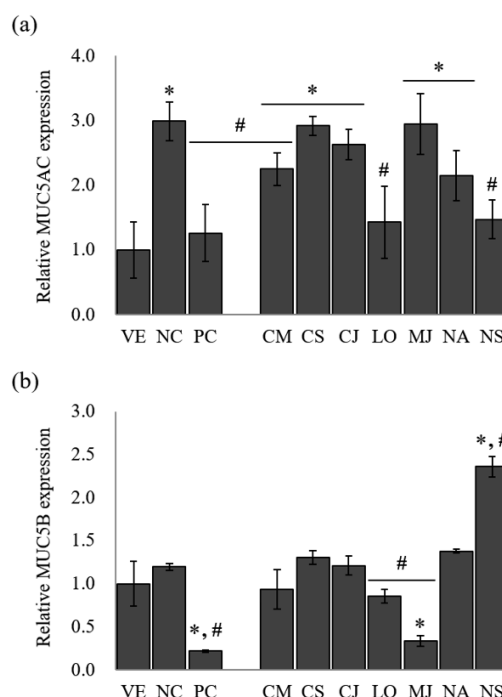


Fig. 3. Effects of seven EOs on LPS-induced mucin production: (a) MUC5AC, (b) MUC5B gene expressions. Values are expressed as the mean \pm SD. * $p < 0.05$ compared with VE; # $p < 0.05$ compared with NC. VE: vehicle, NC: negative control (1 $\mu\text{g}/\text{mL}$ lipopolysaccharide), PC: positive control (5 $\mu\text{g}/\text{mL}$ budesonide), CM: *Cinnamomum camphora*, CS: *Cinnamomum cassia*, CJ: *Cinnamomum japonicum*, LO: *Lindera obtusiloba*, MJ: *Machilus japonica*, NA: *Neolitsea aciculata*, NS: *Neolitsea sericea*, EOs: essential oils, LPS: lipopolysaccharide.

the LPS-stimulated NCI-H292 cells compared to VE. Budesonide, the PC, reduced the relative expression of the mucin gene by 81.7%. Among the seven EOs, only *M. japonica*, *L. obtusiloba*, and *C. camphora* inhibited the MUC5B gene expression. The EOs of *M. japonica* showed a superior inhibitory effect of the MUC5B gene expression by 71.7% reduction than other EOs.

Among the seven EOs, *L. obtusiloba* significantly suppressed MUC5AC and MUC5B expression ($p < 0.05$). *L. obtusiloba* is distributed in the north and south-

east regions of Asia and has been used in traditional Chinese, Korean, and Japanese medicine over centuries (Lim *et al.*, 2016). In Korea and China, it is traditionally used for restoring blood stasis and inflammatory disorders (Kim *et al.*, 2016). Also, the leaves of *L. obtusiloba* are traditionally consumed as both tea and food (Oak *et al.*, 2014). In a previous study, a methanolic extract of *L. obtusiloba* inhibited airway inflammation in a murine model of ovalbumin (OVA)-challenged asthma, suggesting that it may have possible applications in allergic diseases, such as asthma and atopic dermatitis (Lee *et al.*, 2020). Methanolic extract of *L. obtusiloba* consisted of quercetin rhamnoside (26.04 ± 0.05 mg/g) and kaempferol rhamnoside (15.82 ± 0.02 mg/g), and an unknown compound. *L. obtusiloba* extract treatment significantly suppressed the MUC5AC expression in the lung tissues compared with the OVA-challenged asthma model. When *L. obtusiloba* extract (50 or 100 mg/kg) was treated in the OVA-challenged asthma model, MUC5AC expression was decreased by 3.2% and 32.3%, respectively. Compared with the results of this study, it was shown that the mucin-inhibitory effect of *L. obtusiloba* EO was superior to that of the extract. However, the effects of *L. obtusiloba* oil and the active components have not been reported. Table 2 shows that D-limonene (10.27%), β -eudesmol (10.03%) and hedycaryol (6.79%) are the predominant components in the *L. obtusiloba* oil. Limonene was known to be effective in reducing allergic airway inflammation through previous studies (Hirota *et al.*, 2012; Narke *et al.*, 2018). The chemical composition was compared with other Lauraceae family EOs. The portion of limonene was the highest in *L. obtusiloba* EO. Therefore, it is considered that the superior anti-asthmatic activity of *L. obtusiloba* EO is derived from limonene.

4. CONCLUSIONS

Plant secondary metabolites represent a valuable re-

source that can be directly used for drug development or further modified into therapeutic agents. EOs from the Lauraceae family are known to have various bioactivities. This study evaluated the anti-asthmatic activity of EOs from seven species in the Lauraceae family. Among them, the EO from *L. obtusiloba* leaves significantly suppressed the expression of pro-inflammatory cytokines and mucin genes associated with allergic airway inflammation and mucous hyperplasia. These results are suggested that the EO of *L. obtusiloba* leaves can be used as an agent to suppress mucus hypersecretion. However, the active components that inhibit mucus hypersecretion remain to be identified. Further studies are necessary to explore the specific molecular targets of the active components of EOs *in vivo*.

CONFLICT of INTEREST

No potential conflict of interest relevant to this article was reported.

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