Synergistic Antimicrobial Effect of *Lonicera japonica* and *Magnolia obovata* Extracts and Potential as a Plant-Derived Natural Preservative

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Most people use cosmetics to protect their skin. Preservatives are often used to prevent their contamination upon use. There has been a great demand for natural preservatives due to recent reports on the side effects of parabens. Therefore, we evaluated the antimicrobial activities of *Lonicera japonica* and *Magnolia obovata* extracts and determined their potential as natural preservatives. We found that the 50% ethanol extract from *L. japonica* had antibacterial activity only against *S. aureus* and *P. aeruginosa*, while the ethyl acetate fraction showed antimicrobial activity against all six microbial strains tested. On the other hand, the 70% ethanol extract and the ethyl acetate fraction from *M. obovata* showed antimicrobial activity against all six strains. A synergistic effect against *S. aureus*, *B. subtilis*, and *C. albicans* was confirmed when two ethyl acetate fractions having antimicrobial activity against all six strains were used in combination. Synergistic activity against *B. subtilis* was also confirmed through kill-time analysis. High-performance liquid chromatography was performed to identify the components of each extract. Based on the minimum inhibitory concentration and the results of a disc diffusion assay, we confirmed that caffeic acid and luteolin influenced the antimicrobial activity of *L. japonica* and that the antimicrobial activity of *M. obovata* was influenced by the interaction of magnolol and honokiol with other components. Therefore, this study suggests that the combination of *L. japonica* and *M. obovata* extracts may be used as a plant-derived natural preservative.

Keywords: *Lonicera japonica*, *Magnolia obovata*, antimicrobial activity, synergistic effect

Introduction

The skin is the outermost barrier of the human body. It plays a role in protecting the body from external stimuli such as ultraviolet rays, antigens, microorganisms, and chemical substances [1]. The skin preserves the body in various ways by controlling the pH on its surface, moisture content, and through secretion by the sebaceous glands and sweating. If the function of the skin barrier is compromised, pathogens can penetrate the skin and cause many skin diseases [2]. Therefore, people use cosmetics to maintain skin homeostasis and general skin health [3]. As interest in cosmetics has increased, the demand for these products has also grown rapidly. This eventually led to the development of plant-derived and eco-friendly products [4].

According to the United States Federal Food, Drug, and Cosmetic Act, cosmetics are defined as “articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body...for cleansing, beautifying, promoting attractiveness, or altering the appearance.” Cosmetics contain large amounts of water, which could allow the survival of microorganisms and subsequently cause harmful skin diseases, even though their intended use is for skin protection [5]. Therefore, preservatives are added to cosmetics to prevent microbial contamination [6]. However, recent studies have reported that preservatives, especially parabens, may have side effects. Consequently, the demand for plant-derived natural cosmetics has increased, thereby spurring developments in studies on plant-derived preservatives [7, 8].

Lonicera japonica is a semi-evergreen, broad-leaved shrub

that grows in mountains all over Korea. In Chinese medicine, it is known as “geum-eun-hwa”. The stem, flower, and fruit of *L. japonica* have been used for medicinal purposes, for instance, in treating high fever, acute hepatitis, and inflammation. However, eating any part of the plant for a long time is discouraged because it is slightly toxic. Studies on the antioxidant, antibacterial, and anti-inflammatory properties of *L. japonica* extracts have been reported [9–12]. *Magnolia obovata* is a magnolia and a deciduous tree of Japanese and Chinese origin. It is often called magnolia or yellow magnolia. The bark of *M. obovata* is widely used to improve digestion, as a diuretic, and to control vomiting. The bark is used in Chinese medicine as an antioxidant, antibacterial, anti-inflammatory, and antidepressant [13–16].

In this study, we evaluated the antimicrobial activities of *L. japonica* and *M. obovata* extracts against microorganisms regulated in cosmetics according to the Personal Care Products Council Microbiology Guidelines. The six regulated microorganisms used in this study were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus brasiliensis*. We also determined whether the antimicrobial properties of the two extracts could act synergistically. We used the ethyl acetate fractions that exhibited antimicrobial activity against all six strains. The components of each extract were identified through high-performance liquid chromatography (HPLC). In addition, the presence of the active components in each extract was confirmed by evaluating the antimicrobial activity of each component. This study provides insight into the applicability of *L. japonica* and *M. obovata* extracts as natural preservatives.

Materials and Methods

Equipment and Reagents

The UV-visible spectrophotometer used for experiments was a Cary 50 from Varian (Australia). Various solvents such as ethanol, methanol, and ethyl acetate were purchased from Daejung Chemicals & Metals Co. (Korea). The caffeic acid and luteolin used as standards for comparison were purchased from Sigma Chemical Co. (USA). Magnolol and honokiol were purchased from ActivON. *L. japonica* and *M. obovata* were purchased from Gyeongdong market in Seoul, 2017.

Extraction and Fractionation

Four hundred grams of dried *L. japonica* was finely pulverized, immersed in 8 L of 50% ethanol for 24 h, and then filtered. The filtrate was dried under reduced pressure to obtain a powder. The 50% ethanol extract was then fractionated three times with ethyl

acetate, and the ethyl acetate fraction was concentrated to obtain a powder.

Three hundred grams of dried *M. obovata* was finely pulverized, stirred in 3 L of 70% ethanol for 24 h at 30 °C, and then filtered. The filtrate was dried under reduced pressure to obtain a powder. Then, the 70% ethanol extract was fractionated three times with ethyl acetate, and the ethyl acetate fraction was concentrated to obtain a powder.

Microbial Strains and Culture Conditions

The strains used to evaluate the antimicrobial effect of the extracts were *S. aureus* ATCC 6538, *B. subtilis* ATCC 6051, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231, and *A. brasiliensis* ATCC 16404 obtained from the Korea Culture Center of Microorganisms (KCCM, Seoul, Korea). *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *C. albicans* were cultured on Tryptic Soy Agar (Merck, Germany) for 24 h at 37°C after inoculation. *A. brasiliensis* was inoculated onto Potato Dextrose Agar (Merck, Germany) and cultured in a 30°C incubator for 48 h.

Evaluation of Antimicrobial Activity

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). To determine the minimum inhibitory concentration and the minimum bactericidal concentration for each extract, each strain was used at 3 to 5×10^6 colony-forming units (CFU)/ml. Methylparaben was used as a control. The samples were diluted with DMSO using a 2-fold dilution method [17]. Then, 20 µl of the extract, 20 µl of the microbial suspension (inocula), and 160 µl of the medium (bacteria and yeast - Tryptic Soy Broth, mold - Potato Dextrose Broth) were added to the wells on a 96-well plate. The cells were then observed with the naked eye after cultivation (Bacteria and yeast were incubated for 18 h at 37°C and mold was incubated for 48 h at 30°C.), and the concentration at which the microorganisms did not proliferate was determined to be the minimum inhibitory concentration. The concentration at which colonies were not formed upon inoculation of the culture on an agar plate with a sterilized cotton swab was determined to be the minimum bactericidal concentration.

Disc diffusion assay. The antimicrobial activity of each extract and each fraction obtained with different extraction conditions were determined through a disc diffusion assay. The cultured strains were used at $3 \sim 5 \times 10^6$ CFU/ml. Then, 100 µl of each strain was plated using a sterile spreader. Solutions containing 0.05 mg (luteolin) or 5 mg of the samples were absorbed slowly by paper discs (diameter: 8 mm, Toyo Roshi Kaisha Ltd., Japan). The discs were then dried to volatilize the solvent and then incubated with the previously inoculated plates. The clear zone (mm) formed around each disc was measured to compare the antimicrobial activities of the extracts.

Synergistic Antimicrobial Effects of the Extracts

Checkerboard test. The synergistic effect is generally determined

Table 1. HPLC conditions for separation of EtOAc fraction from *L. japonica*.

Conditions of HPLC analysis				
Column	Shim-pack VP-ODS C18 Column (L : 250 mm, LD : 4.6 mm, 5 µm)			
Detector	UVD 170s DIONEX			
Detection wavelength	254–400 nm			
Flow rate	1.0 ml/min			
Injection volume	20 µl			
	Program order	Time (min)	2% AA ¹⁾ in water (%)	0.5% AA ¹⁾ in 50% ACN ²⁾ (%)
Mobile phase conditions for HPLC gradient-elution	1	0	100	0
	2	10	100	0
	3	150	50	50
	4	200	30	70
	5	210	30	70
	6	215	100	0
	7	220	100	0

¹⁾ AA : Acetic acid, ²⁾ ACN : Acetonitrile

in the same manner as the minimum inhibitory concentration, except that 10 µl each of two different samples are mixed [17]. That is, in the checkerboard test, two samples in different concentration are added to each well. Sample A was diluted 2-fold, and then sample B diluted 2-fold was added. The resulting mixture was incubated with one of the six microorganisms. After incubation, the ratio between Sample A and Sample B at which the growth of the microorganism was not observed visually was determined. The fractional inhibition concentration (FIC) and FIC index were then calculated.

$$\text{FIC index} = \text{FIC}_A + \text{FIC}_B$$

$$= \left(\frac{\text{MIC}_{\text{combination AB}}}{\text{MIC}_{\text{agent A}}} \right) + \left(\frac{\text{MIC}_{\text{combination AB}}}{\text{MIC}_{\text{agent B}}} \right)$$

If the FIC index ≤ 0.5, the effects are synergistic; if 0.5 < FIC

index ≤ 1, the effects are additive; if 1 < FIC index ≤ 4 there is no difference between individual and combined effects; and if FIC index > 4, the effects are antagonistic [17].

Kill-time analysis. Kill-time analysis is a method used to measure the change in the number of bacterial colonies in the culture medium upon the addition of the sample over time [17]. The experiment was conducted using a specific sample concentration selected based on the result of the checkerboard test. For the analysis, 0.5 ml of the sample was added to 4.5 ml of the bacterial suspension (10⁵ CFU/ml). This was then incubated at 3-h intervals for 18 hours. Between each interval, the change in the number of bacterial colonies was determined.

Componential Analysis of Each Extract

The ethyl acetate fraction from each of the two extracts was dissolved in 100% ethanol and filtered using a syringe filter

Table 2. HPLC conditions for separation of EtOAc fraction from *M. obovata*.

Condition of HPLC analysis				
Column	Shim-pack VP-ODS C18 Column (L : 250 mm, LD : 4.6 mm, 5 µm)			
Detector	UVD 170s DIONEX			
Detection wavelength	254–400 nm			
Flow rate	1.0 ml/min			
Injection volume	20 µl			
	Program order	Time (min)	DW ¹⁾ (%)	100% ACN ²⁾ (%)
Mobile phase conditions for HPLC gradient-elution	1	0	75	25
	2	40	50	50
	3	60	5	95
	4	70	5	95
	5	75	95	5

¹⁾ DW : Distilled Water, ²⁾ ACN : Acetonitrile

(Millipore, USA, 0.45 µm). The filtered extract solution was then subjected to nonpolar HPLC (C18) analysis. HPLC analysis of the *L. japonica* extract was performed through gradient elution using a 2% aqueous solution of acetic acid and a 50% aqueous solution of acetonitrile containing 0.5% acetic acid. HPLC separation conditions are shown in Table 1. HPLC analysis of *M. obovata* was performed through gradient elution using distilled water and 100% acetonitrile. HPLC separation conditions are shown in Table 2.

Statistical Analysis

All experiments were conducted in triplicate, averaged and presented followed by the standard deviation.

Results and Discussion

Extraction Yields of *L. japonica* and *M. obovata*

The yields of the 50% ethanol extract and the ethyl acetate fraction from *L. japonica* were 4.85% and 0.41%, respectively. The yields of the 70% ethanol extract and the ethyl acetate fraction from *M. obovata* were 14.52% and 4.47%, respectively (Table 3).

Antimicrobial Activity of *L. japonica* and *M. obovata* Extracts

The antimicrobial activities of *L. japonica* and *M. obovata* extracts were evaluated against six strains. MIC and MBC were used as the parameters. The results are shown in Table 4.

The 50% ethanol extract from *L. japonica* exhibited antibacterial activity only against *S. aureus* and *P. aeruginosa*. Its antimicrobial activity was not as high as that of

Table 3. Yields of *L. japonica* and *M. obovata* extractions.

	Solvent	Yield (% w/w)*
<i>Lonicera japonica</i>	50% EtOH extract	4.85
	EtOAc fraction	0.41
<i>Magnolia obovata</i>	70% EtOH extract	14.52
	EtOAc fraction	4.47

*Yield (% w/w) = (weight of dried extract / weight of dried raw material) × 100

methylparaben, which was used as a control. On the other hand, the ethyl acetate fraction from *L. japonica* exhibited antimicrobial activity against all strains. In particular, the activity of the fraction against *S. aureus* and *P. aeruginosa* was higher than that of the control. However, antimicrobial activity of the fraction against *C. albicans* was similar to that of the control.

The 70% ethanol extract and ethyl acetate fraction from *M. obovata* exhibited antimicrobial activity against the six strains. In particular, the antimicrobial activity of the extract against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *C. albicans* was similar to or higher than that of the control. Against all strains except *B. subtilis* and *E. coli*, the antimicrobial activities of the ethyl acetate fraction was found to be about 2 to 4 times higher than those of the 70% ethanol extract.

Synergistic Antimicrobial Effects of *L. japonica* and *M. obovata*

The synergistic antimicrobial effect of the two extracts was evaluated using the ethyl acetate fractions from *L. japonica* and *M. obovata* since these fractions showed high

Table 4. Minimum Inhibitory Concentration (MIC, µg/ml) and Minimum Bactericidal Concentration (MBC) of *L. japonica* and *M. obovata* extracts and fractions.

Strains	MIC (MBC) (µg/ml)					
	Gram positive bacteria		Gram negative bacteria		Yeast	Mold
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. brasiliensis</i>
<i>Lonicera japonica</i>	5000	-	-	5000	-	-
50% EtOH extract	(10000)	(-)	(-)	(-)	(-)	(-)
<i>Lonicera japonica</i>	78	10000	5000	312	1250	10000
EtOAc fraction	(312)	(10000)	(5000)	(1250)	(10000)	(-)
<i>Magnolia obovata</i>	78	2500	2500	1250	1250	10000
70% EtOH extract	(156)	(2500)	(5000)	(1250)	(5000)	(-)
<i>Magnolia obovata</i>	39	2500	2500	625	625	2500
EtOAc fraction	(78)	(5000)	(2500)	(625)	(625)	(-)
Methyl paraben	2500	2500	1250	1250	1250	625
	(5000)	(10000)	(5000)	(1250)	(5000)	(5000)

Methylparaben used as a control. - no inhibition at 10,000 µg/ml.

Fig. 1. Checkerboard test showing the synergistic antimicrobial effects of *L. japonica* and *M. obovata* fractions against six microorganisms.

(A) *S. aureus*, (B) *B. subtilis*, (C) *E. coli*, (D) *P. aeruginosa*, (E) *C. albicans*, (F) *A. brasiliensis*. Colored wells indicate microbial growth and white wells indicate no microbial growth. The number in the well is the FIC index. Wave patterns indicate the synergistic effect (FIC index ≤ 0.5) and dotted patterns mean an addition effect ($0.5 < \text{FIC index} \leq 1$).

antimicrobial activities against all six strains. The synergistic effect was confirmed through the checkerboard test and the FIC index, which were also used to determine whether there is an additive effect or an indifferent effect upon combined use of the extracts. Additionally, kill-time analysis was conducted using the concentrations and combination at which a synergistic effect against *B. subtilis* was observed.

Checkerboard test. The checkerboard test was used to

confirm the synergistic effect of the ethyl acetate fractions from the two extracts, which showed antimicrobial activity against all six strains. The results are shown in Fig. 1. A synergistic effect was observed against *S. aureus*, *B. subtilis*, and *C. albicans*.

A synergistic antimicrobial effect was observed against *S. aureus*; FIC was calculated to be 0.5 at 20 ppm *L. japonica* and 10 ppm *M. obovata*. Additive effects were also observed

at four ratios of the two extracts. Against *B. subtilis*, the FIC value was 0.5 at 2,500 ppm *L. japonica* and 625 ppm *M. obovata*. The results indicate two ratios with synergistic effects and eight with additive effects. Against *C. albicans*, an FIC value of 0.5 was determined at 313 ppm *L. japonica* and 156 ppm *M. obovata*, and the numbers of ratios showing a synergistic effect and an additive effect were 3 and 7, respectively. No synergistic effect was observed against *E. coli* and *A. brasiliensis*, but additive effects were confirmed. Against *P. aeruginosa*, no synergistic nor additive effects were observed. It appears that the combination of the two extracts has weaker antimicrobial activity than each extract only against *P. aeruginosa*. Therefore, we confirmed that a mixture of *L. japonica* and *M. obovata* ethyl acetate fractions exhibits antibacterial synergy against *S. aureus*, *B. subtilis*, and *C. albicans*.

Kill-time analysis. In the previous experiment, we confirmed that the combination of *L. japonica* and *M. obovata* ethyl acetate fractions exhibits antimicrobial synergy against *S. aureus*, *B. subtilis*, and *C. albicans*. Kill-time analysis was conducted against *B. subtilis* since the synergistic and additive effects were highest against this strain. The control group was treated with DMSO only. The concentration of the ethyl acetate fraction from *L. japonica* was 2,500 ppm while the concentration of the ethyl acetate fraction from *M. obovata* was 625 ppm. The combination of *L. japonica* and *M. obovata* extracts was applied to the microbial strain, and the viable cell count was determined at different time points. The results are shown in Fig. 2.

We found that upon treatment with the *L. japonica*

Fig. 2. The synergistic antimicrobial effects of *L. japonica* and *M. obovata* fractions against *B. subtilis* by kill-time analysis.

Control (○) was treated with DMSO only and the concentration of the ethyl acetate fraction from *L. japonica* (□) and *M. obovata* (■) was 2,500 ppm and 625 ppm, respectively. Combination (▲) indicates a mixture of *L. japonica* and *M. obovata* fractions. The experiments were conducted at 3-h intervals for 18 h and in triplicate. Data are presented as the mean ± SD.

extract, the bacterial population increased gradually over time. Addition of the *M. obovata* extract also resulted in a gradual increase in bacterial population over time, but at a slightly lower rate than when *L. japonica* was added. Treatment with either the *L. japonica* or the *M. obovata* extract resulted in lower bacterial counts than the control, suggesting that the extracts had an antibacterial effect. When the *L. japonica* and *M. obovata* extracts were added in combination, the resulting bacterial count was about 10 times lower than when only each of the extracts was applied. This confirms that combining the two extracts enhances their antibacterial effects. Thus, the synergistic antimicrobial effects of *L. japonica* and *M. obovata* against *B. subtilis* was reconfirmed through kill-time analysis.

Fig. 3. The HPLC chromatogram of ethyl acetate fraction of *L. japonica* and the components in detection wavelength range from 254 to 400 nm.

(A) Ethyl acetate fraction, caffeic acid; 1, luteolin; 2. (B) Caffeic acid. (C) Luteolin.

Fig. 4. The HPLC chromatogram of ethyl acetate fraction of *M. obovata* and the components in detection wavelength range from 254 to 400 nm.

(A) Ethyl acetate fraction, honokiol; 1, magnolol; 2. (B) Magnolol and honokiol (standard).

Componential Analysis of *L. japonica* and *M. obovata*

Since we have confirmed that the extracts have antimicrobial activities against the six strains, we used HPLC to determine the effective components of each extract. The results are shown in Figs. 3 and 4.

HPLC analysis of the *L. japonica* ethyl acetate fraction revealed two components, caffeic acid and luteolin. In Fig. 3, a peak for caffeic acid in the *L. japonica* ethyl acetate fraction was confirmed at 67.768 min and a peak for luteolin at 147.756 min. Caffeic acid, a phenolic compound, is mainly found in coffee beans, fruits, and herbs such as thyme, and is known to have antioxidant and anticancer properties [18, 19]. Luteolin, which has a flavone structure, is usually found in peanut shells, parsley, and celery, and is known to have antioxidant, anti-inflammatory, and cytoprotective properties [20–23].

Analysis of the *M. obovata* ethyl acetate fraction revealed two clear peaks that were identified as magnolol and honokiol. In Fig. 4, a peak for honokiol in the *M. obovata* ethyl acetate fraction was confirmed at 33.881 min and a peak for magnolol at 36.491 min. Magnolol and honokiol

Fig. 5. Antimicrobial activities of *L. japonica*, *M. obovata* extracts/fractions and the components against *P. aeruginosa* by disc diffusion assay.

(A) Methylparaben (as a control); 1, *L. japonica* 50% EtOH extract; 2, *L. japonica* EtOAc fraction; 3, caffeic acid; 4, luteolin; 5. (B) Methylparaben (as a control); 1, *M. obovata* 70% EtOH extract; 2, *M. obovata* EtOAc fraction; 3, magnolol and honokiol; 4.

are classified as lignans and are compounds that exist as isomers. These are found mainly in the bark of magnolia trees and are known to possess antioxidant and antibacterial properties [24, 25]. HPLC analysis confirmed the presence of caffeic acid and luteolin in *L. japonica* and magnolol and honokiol in *M. obovata*.

Antimicrobial Effects of the Components of *L. japonica* and *M. obovata* Extracts

The antimicrobial activity of each component of each extract was evaluated, and the relationship between the antimicrobial activity of the extract and that of each component was determined. The antimicrobial activity was evaluated through a disc diffusion assay and the minimum inhibition concentration.

Disc diffusion assay. To evaluate the antimicrobial activity of each component of the *L. japonica* and *M. obovata* extracts, we performed disc diffusion assay. Antimicrobial activity was indicated by the presence of a clear zone around the disc containing the sample. For all samples except luteolin, 5 mg was added onto each disc, while for luteolin, only 0.05 mg was added.

As shown in Fig. 5, the results were most effective against *P. aeruginosa*, with the clear zones around the discs containing the 50% ethanol extract and the ethyl acetate

Table 5. Minimum Inhibitory Concentration (MIC, ug/ml) of the components of *L. japonica* and *M. obovata* extracts.

Strains	MIC (ug/ml)					
	Gram positive bacteria		Gram negative bacteria		Yeast	Mold
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. brasiliensis</i>
Methyl paraben	1250	1250	1250	1250	1250	625
Caffeic acid	312	5000	5000	2500	5000	> 10000
Luteolin	25	200	200	50	200	200
Magnolol, Honokiol	31.2	10000	10000	1250	5000	39

Methylparaben used as a control.

fraction from *L. japonica* measuring 8 mm and 9 mm in diameter, respectively. These activities were lower than that of the control, methylparaben (18 mm). Caffeic acid, one of the components of the *L. japonica* extract, produced a 10-mm clear zone, while luteolin did not inhibit bacterial growth. Similarly, the 70% extract and the ethyl acetate fraction from *M. obovata* showed inhibitory activities, as indicated by the clear zones (11 mm and 10 mm, respectively) around the corresponding discs, which were lower than that of the control, methylparaben (16 mm). Magnolol and honokiol, which are the main components of the extract, did not produce clear zones.

Minimum inhibitory concentration (MIC). To accurately evaluate the antimicrobial activity of each component of the *L. japonica* and *M. obovata* extracts, the minimum inhibitory concentration was determined. The results are shown in Table 5.

Caffeic acid showed high antimicrobial activity against *S. aureus* and *P. aeruginosa*, and low antimicrobial activity against *B. subtilis*, *E. coli*, and *C. albicans*. No antimicrobial activity against *A. brasiliensis* was detected even at high concentrations of the components. On the other hand, luteolin exhibited antimicrobial activity against all strains at low concentrations and showed excellent antimicrobial activity especially against *S. aureus* and *P. aeruginosa*. This suggests that the no inhibition by luteolin was detected in the disc diffusion assay because the concentration of the compound used was too low. As shown in Table 4, the 50% ethanol extract from *L. japonica* exhibited antimicrobial activity against *S. aureus* and *P. aeruginosa*. This can be explained by the activity of caffeic acid, which has relatively high polarity, since the extract and this component had antibacterial activities against the same strains. On the other hand, the antimicrobial activities of the ethyl acetate fraction from *L. japonica* were observed against six strains, which is expected as a result of the influence of luteolin, a nonpolar component.

Magnolol and honokiol showed antimicrobial activity

against all strains, especially *S. aureus*, *P. aeruginosa*, and *A. brasiliensis*. These results suggest that the antimicrobial effects of the *M. obovata* extract can be attributed to the presence of magnolol and honokiol. In addition, the antimicrobial effects of the extract may be a result of the interaction between magnolol, honokiol, and other ingredients since the antimicrobial effects of the extracts are superior to those of each component.

In this study, we evaluated the antimicrobial activities of *L. japonica* and *M. obovata* extracts in order to examine their potential as natural preservatives. We found that the 50% ethanol extract from *L. japonica* had antimicrobial activities against *S. aureus* and *P. aeruginosa*, and the ethyl acetate fraction had antimicrobial activities against all six strains tested. On the other hand, the 70% ethanol extract and ethyl acetate fraction from *M. obovata* showed antimicrobial activities against all six strains, with particularly high activities against *S. aureus*, *P. aeruginosa*, and *C. albicans*.

We also evaluated the synergistic antimicrobial activity of the ethyl acetate fractions from *L. japonica* and *M. obovata*, both of which showed antimicrobial activity against all six strains. We showed that the two fractions acted in synergy against *S. aureus*, *B. subtilis*, and *C. albicans*. This was reconfirmed through kill-time analysis against *B. subtilis*.

To determine the extract components that have antimicrobial activity, we performed HPLC analysis and detected the presence of caffeic acid and luteolin in the *L. japonica* extract and magnolol and honokiol in the *M. obovata* extract. The results of the disc diffusion assay and the minimum inhibitory concentration indicate that the antimicrobial activity of *L. japonica* is influenced by caffeic acid and luteolin. On the other hand, the antimicrobial activity of *M. obovata* was a result of the interaction among magnolol, honokiol, and other compounds. Moreover, further research, such as on the mechanisms underlying the antimicrobial activities of the two extracts and on a challenge test (preservative effectiveness test) of products containing the extracts, should be conducted. Thus, this

study suggests that a combination of the *L. japonica* and *M. obovata* extracts may be applicable as a plant-derived natural preservative.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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