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Antimicrobial activity of essential oil of *Lindera umbellate* var. Membrancea

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Abstract
Lindera umbellate var. Membrancea is endemic throughout Japan and has long been used as a fragrant wood and for crafting. Examination of its chemical composition identified linalool as the most abundant constituent. In activity testing by micro-broth dilution method, the minimum inhibitory concentration (MIC) of essential oil of *L. umbellate* against *Staphylococcus aureus* FDA209P and *Escherichia coli* NIHJ JC2 was 50 and 800 mg/ml, respectively. With essential oil at the respective MICs at broth dilution with shaking method, the growth of *S. aureus* was not observed for 15 h and the growth of *E. coli* was delayed for 23.4 h. We showed that essential oil of *L. umbellate* effectively inhibited the growth of bacteria.

Keywords: *Lindera umbellate* var. Membrancea, essential oil, antibacterial activity, growth curve

Introduction
Lindera umbellate var. Membrancea is an endemic plant species found throughout Japan [1]. The wood is fragrant, and the essential oil of *L. umbellate* var. Membrancea exhibits a sedative effect [2]. Essential oil from the species is utilized for its anti-inflammatory properties to relieve gastrointestinal upset and skin problem [3]. Owing to its hardness and fragrance, wood from *L. umbellate* var. Membrancea has long been used for making toothpicks. It is also said that these toothpicks can help prevent tooth decay caused by bacteria. In the present study, we examined the antibacterial activity of essential oil of *L. umbellate* var. Membrancea.

Materials and Methods
Plant material and extraction
Essential oil of Lindera umbellate var. Membrancea used in this study was prepared by Rapias Electronics Co., Ltd. (Iwate, Japan) using branches and leaves collected at Iwate Prefecture, which is located in the northern part of Japan, and extracted using a steam distillation method.
The constituents of L. umbellate essential oil were analyzed by gas chromatograph and time of flight mass spectrometry (GC-TOFMS) using a Master GC-TOFMS (DANI Instruments, S.a.A., Milan, Italy) equipped with a DB-WAX column (60 m x 0.25 mm i.d., Agilent Technology, Santa Clara, CA, USA) and a flame ionization detector (FID). The oven temperature was programmed to increase from 40 to 250°C at a rate of 3°C/min with a hold at 250 °C for 2 h. The injector and FID were maintained at 240°C.
Essential oil constituent ratios were determined based on peak area of GC chromatograms.

Bacterial cells
Staphylococcus aureus FDA209P and Escherichia coli NIHJ JC2 were used as standard strains and were cultured in brain heart infusion broth and Mueller-Hinton broth (Becton Dickinson and Company, Franklin Lake, NJ, USA), respectively.

Antibacterial activity assay
Antibacterial activity expressed as the minimum inhibitory concentration (MIC) and the delay in proliferation were determined according to the micro-broth dilution and broth dilution with shaking methods, respectively. MIC was defined as the lowest concentration of the essential oil at which visible growth in broth culture was not observed for 24 h [4].
Turbidity was measured as the optical density (O.D.) by broth dilution with shaking method using a biophotorecorder TN-1512 (Toyo Seisakusho Kaisya Ltd., Tokyo, Japan) [5]. The delay in proliferation was determined as difference in time from when O.D. reached to 1.0 between the condition with and without the test substance in broth.

Dimethyl sulfoxide was used to prepare stock solution of *L. umbellate* var. Membrancea essential oil and its constituents.

Results and Discussion

The major constituents of L. umbellate essential oil are shown in Table 1. The highest constituent was linalool that was similar result to prior research [6]. Although the rankings differed, 1, 8-cineol, geranyl acetate, and dihydrocarvone were major constituents.

Table 1: Major constituents of Lindera umbellate var. Membrancea essential oil

Constituent	Ratio (%)
linalool	40.3
1,8-cineole	12.2
geranyl acetate	5.2
dihydrocarvone	5.4
borneol	4.7
limonene	4.3
a-pinene	3.8
a-terpineol	2.4
a-cymene	2.3
terpinen-4-ol	2.1
camphene	2.1
3-carene	2.1
bornyl acetate	1.2
linalool oxide	0.8
b-pinene	0.7
myrcene	0.5
geraniol	0.4
camphor	0.3
a-phellandrene	0.2
terpinyl acetate	0.2

The MIC of essential oil of L. umbellate against S. aureus and E. coli was 50 and 800 mg/ml, respectively (Table 2). While the essential oil of L. umbellate has clear antibacterial activity against both bacterial strains tested, it was more effective against S. aureus than E. coli. The MIC determined by the micro-broth dilution method indicates the concentration necessary to suppress bacterial growth for 24 h but it does not provide information about the effect of lower concentration essential oil on bacterial growth. To gain more insight on the effect of essential oil on bacterial growth, the turbidity of bacterial suspensions with and without various concentrations of essential oil up to 800 mg/ml was recorded over time.

Table 2: MIC values for Lindera umbellate var. Membrancea essential oil against S. aureus and E. coli

Bacteria strain	MIC (mg/ml)
S. aureus	50
E. coli	800

The time course of turbidity for various concentrations of essential oils indicates that the turbidity went below zero for essential oil at 400 and 800 mg/ml, indicating a dissolving process. In the absence of essential oil, turbidity reached 1.0 at 16.7 h from the start of incubation with S. aureus (Figure 1) but with the addition of 50 mg/ml of essential oil, the turbidity reached 1.0 at 31.3 h, indicating a delay in growth of 15 h. For concentrations of essential oil over 100 mg/ml, bacterial growth was not observed. Therefore, the essential oil of L. umbellate showed effective antibacterial activity against S. aureus.

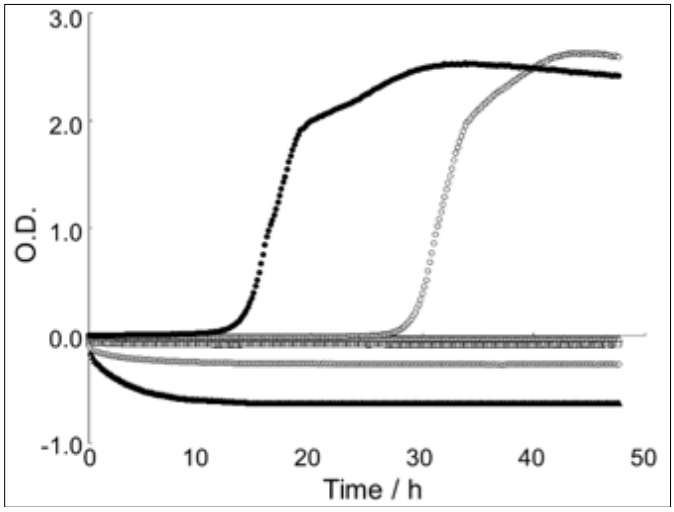

Fig 1: Effect of Lindera umbellate var. Membrancea essential oil on the growth curve of S. aureus FDA209P

Closed triangles: 800 mg/ml, open diamonds: 400 mg/ml, open squares: 200 mg/ml, open triangles: 100 mg/ml, open circles: 50 mg/ml, closed circles: control.

Figure 2 shows the time course of turbidity of bacterial suspension of E. coli and the essential oil with various concentration. Turbidity reached 1.0 at 11.3 h from the start of incubation without essential oil. This phenomenon was considered that the essential oil inhibited the growth of E. coli. For E. coli, essential oil suppressed the maximum turbidity. The slope of exponential growth phase became smaller with increasing the concentration of the essential oil, indicating that the essential oil decreased the growth rate of E. coli.

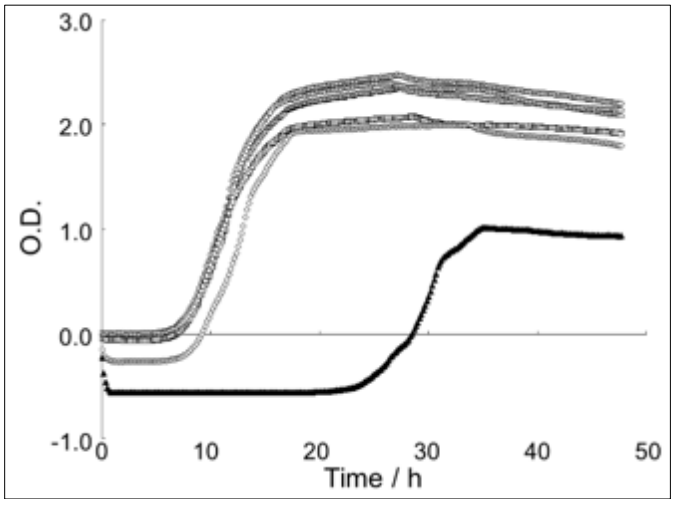

Fig 2: Effect of Lindera umbellate var. Membrancea essential oil on the growth curve of E. coli NIHJ JC2

Closed triangles: 800 mg/ml, open diamonds: 400 mg/ml, open squares: 200 mg/ml, open triangles: 100 mg/ml, open circles: 50 mg/ml, closed circles: control.

Conclusion

The essential oil of Lindera umbellate var. Membrancea showed antibacterial activity against gram positive and gram-negative bacteria with more effective activity against gram positive bacteria. The essential oil of Lindera umbellate is reportedly safe for humans [7]. This essential oil might be

useful in oral care and facial care products since gram-positive bacteria included caries-causing bacteria and facial skin problem-causing bacteria.

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