Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(1): 205-207
Received: 02-11-2022
Accepted: 06-12-2022

Yoshihiro Inoue
Showa Pharmaceutical University, Machida, Tokyo, Japan

Souichi Ikeno
Showa Pharmaceutical University, Machida, Tokyo, Japan

Corresponding Author:
Yoshihiro Inoue
Showa Pharmaceutical University, Machida, Tokyo, Japan

Synergistic effect of constituents of essential oil of *Lindera umbellata* var. Membrancea on antibacterial activity

Yoshihiro Inoue and Souichi Ikeno

DOI: https://doi.org/10.22271/phyto.2023.v12.i1c.14577

Abstract
Lindera umbellata var. Membrancea (Kuromoji) is a wild plant that grows all over Japan. Various methods have been proposed for utilizing the characteristics of its essential oils. The chemical composition of the essential oil of *L. umbellate* var. Membrancea and its antibacterial activity against gram-positive bacteria have been reported. To broaden the range of applications, we aimed to investigate the correlation between the oil constituents and their activity in various combinations. Borneol was found to be an important constituent for antibacterial activity. It is suggested that the presence of five major constituents, namely, linalool, 1, 8-cineole, geranyl acetate, dihydrocarvone, and borneol, caused a significant synergistic increase in the antibacterial activity.

Keywords: Essential oil, *Lindera umbellata* var. Membrancea, antibacterial activity, synergistic effect

Introduction
The use of forest resources, which are abundant in our immediate surroundings, has been proposed to improve the functioning of our living environment and quality of life. *Lindera umbellata* var. Membrancea (Kuromoji) is a wild plant that grows throughout Japan [1]. Various uses of its essential oil are being explored. We previously reported the chemical composition of the essential oil of *L. umbellate* var. Membrancea and its effective antibacterial activity against gram-positive bacteria [2]. However, the effect of the oil constituent components on its antimicrobial activity remains unclear. Therefore, this study aimed to clarify the relationship between the antimicrobial activity of the oil and its constituents.

Materials and Methods
General
Linalool, 1, 8-cineole, geranyl acetate, dihydrocarvone, borneol, and dimethyl sulfoxide were purchased from Nihon Kasei Co. Ltd.

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

Antibacterial activity assay
Antibacterial activity was expressed as the minimum inhibitory concentration (MIC) according to the micro-broth dilution method [3]. The compound to be tested was added to aliquots of BHI or MH broth (100 mL). An aliquot of an overnight culture of *S. aureus* or *E. coli* (ca. 1 x 10^5 cfu/mL) was added to each sample. Each culture was incubated in air without shaking at 37°C for 24h.
Dimethyl sulfoxide was used to prepare a stock solution of *L. umbellate* var. Membrancea essential oils and their constituents.

Results and Discussion
In a previous study, we reported the ratio of the constituents of the essential oil of *L. umbellate* var. Membrancea and their antibacterial activity against *S. aureus* and *E. coli* [2]. (Table 1) The major constituents were linalool and 1, 8-cineole. This finding is similar to the results of prior research [4, 5]. To investigate the relationship between antibacterial activity and the constituents, the antibacterial activities of each major constituent was observed (Table 2).

Linalool, 1, 8-cineole, geranyl acetate, dihydrocarvone, and borneol were selected as the major constituents because their concentrations in the oil were over 5% (mass percent).

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

Constituents	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

Table 2: MIC of a single constituent of *Lindera umbellata* var. Membrancea essential oil

Constituent	against *S. aureus*	against *E. coli*
Linalool	800	>800
1,8-cineol	>800	>800
Geranyl acetate	800	>800
Dihydrocarvone	>800	>800
Borneol	400	800

Linalool, geranyl acetate, and borneol showed activity against *S. aureus*, whereas borneol activity was most intense. The antibacterial activity of borneol against *E. coli* was also observed. None of the constituents demonstrated the activity equivalent to that of the essential oil alone.

The effect of the constituents' combinations on the antibacterial activity was investigated. Under the experimental conditions used, the highest total mass concentration of the constituent mixture was set to 800 mg/mL, which was the highest concentration of the essential oil at the MIC.

Table 3 shows the antibacterial activity of a mixture of the two constituents. Under the experimental conditions of the combination of the two constituents, the highest concentration was 400+400 [mg/mL]. All combinations effectively inhibited bacterial cell growth. The two constituent mixtures more strongly inhibited the growth of *S. aureus* than that of *E. coli*. The combination of linalool and borneol strongly inhibited the growth of both *S. aureus* and *E. coli*. The MIC of the combination of linalool and borneol against *E. coli* was 400 + 400 [mg/ml]. When the MIC of the essential oil from *Lindera umbellata* var. Membrancea was 800 mg/mL, the concentrations of linalool and borneol were 320 mg/mL and 37.6 mg/mL, respectively. Linalool and borneol play important roles in antibacterial activity against *E. coli*. However, these two constituents could not reach the activity of the essential oil of *Lindera umbellata* var. Membrancea. The essential oil of *Lindera umbellata* var. Membrancea was highly effective against *S. aureus*, whereas activities of a single constituent and the combination of two constituents were much lower than the activity of the essential oil.

Table 3: MIC of the combination of two constituents of *Lindera umbellata* var. Membrancea essential oil

Combination of constituents	against *S. aureus*	against *E. coli*	[mg/mL]
Linalool	+1,8-cineole	400+400	800+800
	+Geranyl acetate	400+400	800+800
	+Dihydrocarvone	400+400	800+800
	+Borneol	200+200	400+400
1,8-cineole	+Geranyl acetate	400+400	800+800
	+Dihydrocarvone	400+400	800+800
	+Borneol	400+400	800+800
Geranyl acetate	+Dihydrocarvone	400+400	800+800
	+Borneol	400+400	800+800
Dihydrocarvone	+Borneol	400+400	800+800

To further examine the relationship between the antibacterial activity of the essential oil of *L. umbellate* var. Membrancea and its constituents, the number of constituents to be combined was increased.

Table 4 shows the results for the combination of three major constituents. Under the experimental conditions used, the highest concentration was 266+266+266 [mg/mL] as the total concentration was set to 800 mg/mL, which was the highest concentration of the essential oil. All combinations were effective in inhibiting the growth of *S. aureus*. The strength of the inhibition increased when borneol was included in the combination treatment. However, the antibacterial activity of any combination of three major constituents did not exceed those of the essential oil.

Table 4: MIC of the combination of three constituents of *Lindera umbellata* var. Membrancea essential oil

Combination of constituents		against *S. aureus*	[mg/mL]
Linalool	+1,8-cineole	+Geranyl acetate	266+266+266
		+Dihydrocarvone	266+266+266
		+Borneol	266+266+266
	+Geranyl acetate	+Dihydrocarvone	266+266+266
		+Borneol	133+133+133
	+Dihydrocarvone	+Borneol	133+133+133
1,8-cineole	+Geranyl acetate	+Dihydrocarvone	266+266+266
		+Borneol	133+133+133
	+Dihydrocarvone	+Borneol	133+133+133
Geranyl acetate	+Dihydrocarvone	+Borneol	133+133+133

The results for the four combined constituents are shown in Table 5. The highest concentrations were 200+200+200+200 [mg/mL]. As with the combinations of the three constituents, antibacterial activities were investigated for all combinations. The borneol combinations showed strong activity, but the strength was not close to that of the essential oil.

Table 5: MIC of the combination of four constituents of *Lindera umbellata* var. Membrancea essential oil

Combination of constituents against *S. aureus* [mg/mL]
Linalool+1,8-cineole+geranyl acetate+dihydrocarvone 200+200+200+200
Linalool+1,8-cineole+geranyl acetate+borneol100+100+100+100
Linalool+1,8-cineole+dihydrocarvone+borneol 100+100+100+100
Linalool+geranyl acetate+dihydrocarvone+borneol100+100+100+100
1,8-cineole+geranyl acetate+dihydrocarvone+ borneol100+100+100+100
Linalool+1,8-cineole+geranyl acetate+dihydrocarvone+borneol10 +10+10 +10

The results for the five combined constituents are shown in Table 5. The highest concentrations were 160 + 160 + 160 + 160 + 160 [mg/mL]. The presence of all five major constituents dramatically increased antibacterial activity, and the strength reached that of the essential oil.

Therefore, we showed that borneol plays an important role in the antibacterial activity of the essential oil of *L. umbellate* var. Membrancea against *S. aureus*, and the presence of all five major oil constituents dramatically increased the mixture activity.

Conclusions

The antibacterial activity of the essential oil of *L. umbellate* var. Membrancea was greatly supported by Borneol against both gram-negative and gram-positive bacteria. The addition of linalool increased the activity of Borneol against gram-negative bacteria. The presence of five major constituents dramatically increased the mixture activity against gram-positive bacteria. This enhancement was synergistic and not additive.

Our study is expected to provide new ways of using essential oils in the medical field if the mechanism of the substantial increase in the activity against gram-positive bacteria is elucidated.

References

1. Nakamura M, Nanami S, Okuno S, Hirota S, Matsuo A, Suyama Y, *et al*. Genetic diversity and structure of apomictic and sexually reproducing *Lindera* species (Lauraceae) in Japan. Forests. 2021; 12: 227-239.
2. Inoue Y, Chiba R. Antimicrobial activity of essential oil of *Lindera umbellata* var. Membrancea, submitted
3. National committee for clinical laboratory standard. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-fifth edition, Approved standard.: Wayne, PA, USA, 2000. M7-A5
4. Nanashima N, Kitajima M, Takamagi S, Fujioka M, Tomisawa T. Comparison of chemical composition between Kuromoji (*Lindera umbellata*) essential oil and hydrosol and Determination of the deodorizing effect. Molecules. 2020;25:4195-4200.
5. Kitajima M, Miura M, Nanashima N, Tominsawa T, Takamagi S, Fujioka M, *et al*. Psychological and antibacterial effects of footbath Using the *Lindera umbellata* essential oil. Molecules. 2021;26:5128-5135.