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Yoshihiro INOUE
Showa Pharmaceutical University, Machida, Tokyo, Japan

Ryoko CHIBA
Nohon Pharmaceutical University, Saitama, Japan

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

Antibacterial activity of *Cryptomeria japonica* (Sugi) essential oil constituents

Yoshihiro INOUE and Ryoko CHIBA

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Abstract
The essential oil of *Cryptomeria japonica* (Sugi) is of interest for the reasons of medical effectiveness. We reported the antibacterial activity of the essential oil. But it remains unclear that the relationship between the antibacterial activity and the constituents. The results of antibacterial activity measurement suggested that β–eudesmol was important constituent to inhibit the growth of *S. aureus* and terpinene-4-ol and limonene was necessary combination to suppress the growth of *E. coli*. It is also found that the antibacterial activity essential oil of *Cryptomeria japonica* appears to be due to synergistic effects of the constituents.

Keywords: *Cryptomeria japonica*, antibacterial activity, synergistic effect

Introduction
Cryptomeria japonica (Sugi) is endemic species of Japan plant and widely distributed in Japan. It has also been widely introduced as a forest resources. It has been widely used as building materials because of its ease of processing and durability. The leaves of *Cryptomeria japonica* contain bioactive compounds associated with essential oil and flavones. *Cryptomeria japonica* has attracted a great deal of interest in the field of aromatherapy and medical usage.
In previous study, we reported the antibacterial activity of the essential oil against gram positive and negative bacteria.1) Essential oil consisted with many constituents. If we could clarify which constituents play an important role to the antibacterial activity, it may lead to new uses and new medicines. The expertise would give us effective and safety usage of the essential oil for our good health. In this study, we investigated that the role of constituents in the antibacterial activity of essential oil of *Cryptomeria japonica*.

Materials and Method
General
Essential oil of *Cryptomeria japonica* was obtained by using steam distillation method with branch and leaves collected in Akita prefecture and Iwate prefecture which is norther part of Japan [1].
Kaur-16-ene, β–eudesumol, α–pinene, terpinene-4-ol, limonene, γ–terpinene and dimethyl sulfoxide were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Bacterial cell
Staphylococcus aureus FDA209P and *Escherichia coli* NIJH JC2 were incubated in Brain Heart Infusion (BHI) broth and Luria-Bertani (LB) broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), respectively.

Antibacterial activity assay
Minimum inhibitory concentration (MIC) was determined by micro broth dilution method [2, 3]. BHI broth and Mueller-Hinton (MH) broth was used in determining MIC for *S. aureus* and *E. coli*, respectively.
Dimethyl sulfoxide was used to dissolve hydrophobic substances into broth.
Double dilution series of constituents of essential oil were prepared with broth and bacterial suspension was inoculated as initial bacterial content was 10^4 cfu/ml. After 24 hour incubation at 27 °C, MIC was determined as the lowest concentration at which no increase in turbidity was observed.

Results and Discussion

Table 1 was the list of constituents of two essential oil of *Cryptomeria japonica* that we obtained.1) Essential oil of *Cryptomeria japonica* harvested in Akita Pref. showed the antibacterial activity as MICs for *S. aureus* and *E. coli*. Essential oil of *Cryptomeria japonica* harvested in Iwate Pref. showed similar antibacterial activity especially for *S. aureus*. To determine which constituent was the important one to show the activity, antibacterial activity of constituents which was major and contained in both areas harvested was investigated. (Table 2).

Except the case of β–eudesmol against *S. aureus*, the antibacterial activity was not observed in the experimental condition used in this study. Essential oil was mixture of various constituents. Then, the antibacterial activity of mixture of constituents was observed. The antibacterial activity of the mixture of two constituent listed in Table 2 against *S. aureus* was shown in Table 3.

When β–eudesmol was included, MIC value reached to be 50 μg/ml. Considering the content of β–eudesmol in the essential oil, the concentration of β–eudesmol was about 12 μg/ml. These results suggested that β–eudesumol was important constituent and was not responsible for the antibacterial activity of the essential oil. In the case of a-pinen plus terpinene-4-ol, MIC value decreased to be 200 μg/ml. Although both a-pinene and terpinene-4-ol did not show the activity, co-existence of the two constituents inhibited the growth of *S. aureus*. For the other combination, the inhibition in the growth was observed even the single use was not effective.

Antibacterial activity of the mixture of constituent of the essential oil was observed against *E. coli*. (Table 4) The combination of terpinene-4-ol and limonene showed the antibacterial activity. MIC value of essential oil was 200 μg/ml. In the essential, terpinene-4-ol and limonene was included of about 12 and 8 μg/ml, respectively. The activity was obvious, but not all of the activity of the essential oil. For many combination, the activity was observed though both constituent did not show the activity at single use.

Conclusions

When the essential oil showed the antibacterial activity, it is suggested that important constituent is existed. β–eudesmol was the one against *S. aureus* and terpinene-4-ol and limonene were the ones against *E. coli*. It is also revealed that the antibacterial activity of essential oil was the results of a synergistic effect. It is necessary to elucidate the mode of action of the antibacterial activity of the essential oil for effective and safety use to our health.

Table 1: Constituents of essential oil of *Cryptomeria japonica*

Area of forest harvesting		
Constituent	**Akita Pref.**	**Iwate Pref.**
a-pinene	23	10
b-eudesmol	6	12
terpinene-4-ol	5	6
limonene	7	4
Kaur-16-ene	6	3
g-terpinen	1	6
sabinene	9	1
elemol	7	
cadenene	1	
a-bulnesene	---	10
others	33	49

Content ratio was determined by GC peak area

Table 2: Antibacterial activity of constituents of the essential oil of *Cryptomeria japonica*

MIC (μg/ml)		
Constituents	**Against *S. aureus***	**Against *E. coli***
α–pinene	>400	>400
β–eudesmol	50	>400
terpinene-4-ol	>400	>400
limonene	>400	>400
kur-16-ene	>400	>400
γ–terpinen	>400	>400

Table 3: Antibacterial activity of mixture constituent against *S. aureus*

Combination of constituent		MIC (μg/ml)	Combination of constituent		MIC (μg/ml)
β–eudesumol	+ α-pinen	50+50	α–pinen	+ kaur-16-ene	400+400
	+ terpinene-4-ol	50+50	terpinen-4-ol	+ limonene	400+400
	+ limonene	50+50		+ γ–terpinen	400+400
	+ γ–terpinen	50+50		+ kaur-16-ene	400+400
	+ kaur-16-ene	50+50	limonene	+ γ–terpinene	400+400
α–pinen	+ terpinene-4-ol	200+200		+ kaur-16-ene	400+400
	+ limonene	400+400	γ–terpinen	+ kaur-16-ene	400+400
	+ γ–terpinen	400+400			

Table 4: Antibacterial activity of mixture constituent against *E. coli*

Combination of constituent		MIC (μg/ml)	Combination of onstituent		MIC (μg/ml)
β–eudesumol	+ α–pinen	400+400	α–pinen	+ kaur-16-ene	400+400
	+ terpinene-4-ol	400+400	terpinen-4-ol	+ limonene	200+200
	+ limonene	400+400		+ γ–terpinen	400+400
	+ γ–terpinen	>400+>400		+ kaur-16-ene	400+400
	+ kaur-16-ene	400+400	limonene	+ γ–terpinene	400+400
α–pinen	+ terpinene-4-ol	400+400		+ kaur-16-ene	400+400
	+ limonene	>400+>400	γ–terpinen	+ kaur-16-ene	400+400
	+ γ–terpinen	>400+>400			

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