

Antibacterial Effect of Lavender (*Lavandula*) Flavor (Volatile)

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Abstract: The objective of this study was to test the essential oil-emitted flavor (volatile) of lavender by bacteria killing potency using *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), enterohemorrhagic *E. coli* O157:H7, *Pseudomonas aeruginosa* and *Candida albicans*. Antibacterial activity was assessed by creating of the bacterial growth curve in the liquid medium cultivation and the growth inhibition on the agar plate cultivation. Citronellal, one of compounds in *Rosa rugosa* oil, was used as a positive control for comparison in activity. The results showed that lavender and citronellal flavor (volatile) or oil respectively demonstrated bacteria killing effect in both analytical manners. However, *P. aeruginosa* resisted bacteria killing potency of lavender (citronellal) oil or oil-emitted flavor. It could be concluded that both lavender oil-emitted flavor and oil showed bacteria killing potency. Lavender oil (or oil-emitted flavor) will be expected to apply for the patient waiting room to disinfect in air along with the mental relaxation of the patient.

Key words: Lavender flavor (volatile), citronellal flavor (volatile), bacteria killing potency, growth curve of bacteria.

1. Introduction

A historically huge outbreak of food poisoning caused by enterohemorrhagic *Escherichia coli* O157:H7 occurred in Japan 1996 and ended with the 12 deaths out of the 20,000 patients at the end of this year. The authors immediately initiated a search to find functional foods with anti-bacterial activity. During studies, the authors observed an interesting event that bacteria did not grow on agar culture medium, when exposed to odor from the Hiba tree/Japanese cypress, even the tests were repeated. The authors speculated that this phenomenon was probably due to the effect of flavor (volatile) released from the Hiba tree. To confirm a strange phenomenon, two types of plant oils (flavor) of the *Lavandula* and *Rosa rugosa* were subjected to the studies.

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Lavandula (generally called lavender) is flowering plants in the mint family, Lamiaceae [1]. It is found from Cape Verde and the Canary Islands, Southern Europe across to Northern and Eastern Africa, the Mediterranean, Southwest Asia to Southeast India including Japan (Fig. 1). Many of them are cultivated extensively in temperate climate as ornamental plants for garden and landscape, as culinary herbs and also commercially for the extraction of essential oils. The most widely cultivated species, *Lavandula angustifolia*, is often referred to as lavender, and there is a colour named for the shade of the flowers of this species.

Rosa rugosa (rugosa rose, Japanese rose or Hamamas rose in Japan) is a species of rose native to Eastern Asia in Northeastern China, Japan, Korea and Southeastern Siberia, where it grows on the coastal sites and often on sand dunes. It has single, fragrant blossoms opening from pale pink buds, its foliage is dark green and it forms large, brick-red rosehip [2].

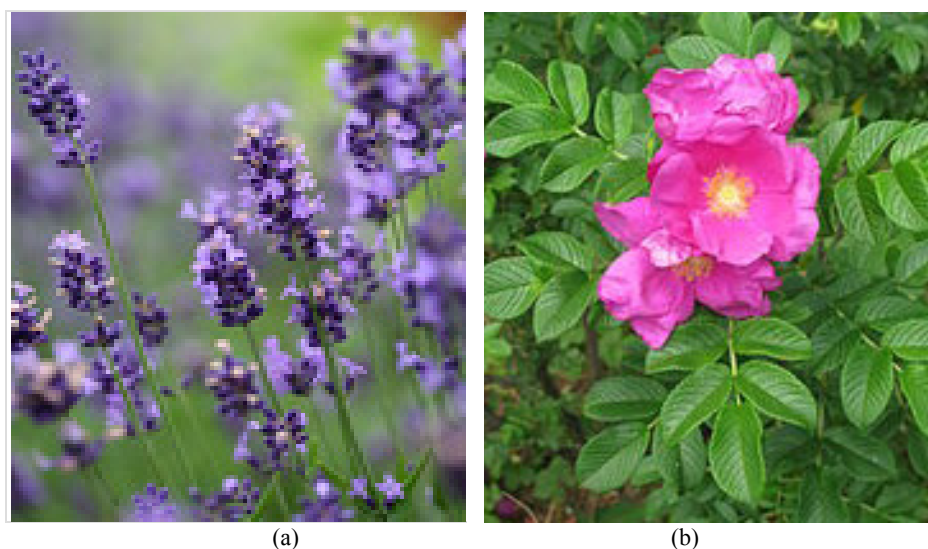


Fig. 1 Lavender (a) and *Rosa rugosa* with flowers (b).

Flowers are large, fragrant roses and extremely disease resistant. Many have a spectacular show of red hips after flowering rich of vitamin C.

These kinds of plants are well known in Japan by producing smell or odor (flavor) caused by volatile consisting with chemical compounds. It is generally accepted that flavor affects both the sense of taste and smell, whereas fragrance affects only smell. Flavor tends to be naturally occurring, and fragrance tends to be synthetic [3]. Further aroma compounds can be found in food, wine, spices, perfumes, fragrance oils, and essential oils. Also, many of the aroma compounds play a significant role in the production of flavor, which are used in the food service industry to flavor, improve and generally increase the appeal of their products. Many experimental reports were recognizable on antibacterial effect of lavender oil [4, 5], but very few were admitted in the form of the scientific descriptions. In this paper, the authors presented the bacteria killing effect of the lavender or *Rosa rugosa* citronellal-emitted flavor (volatile).

2. Materials and Methods

2.1 Extraction of Essential Oil from Lavender (*Lavandula*)

Lavender flowers collected in Aomori, Japan were

hydro-distilled as a standard extraction method. Briefly lavender (petals) was distilled for 6-7 h, followed by extraction of the essential oil with 2 L of floral water (hydrosol). Floral water was then extracted with hexane, diethyl ether and ethyl acetate solvent [6], and stored them in refrigerator until usages. Citronellal, component of *Rosa rugosa*, was used as a reference to compare with that of lavender extracts.

2.2 Composition Analyses of Lavender Extracted Oil

Essential oil was analyzed by the flame ionization detector-gas chromatography (G-5000A Hitachi, Tokyo, Japan). Briefly, a 60 mL/min nitrogen carrier flow rate was passed through a poly (alkylene glycol) column (30 m × 0.25 mm) using a 10 °C/min gradient that began with 5 min hold at 40 °C and ended upon reaching at 200 °C. Mass spectrometer-gas chromatography (GC-MS) analysis was conducted on a JEOL Q1000GC-Mk II mass spectrometer (Japan Electron Optics Lab. Co. Ltd.).

2.3 Target Bacteria Used for Test of Bacteria Killing Potency

Target pathogens used were *E. coli* (ATCC 25922), methicillin-resistant *Staphylococcus aureus* (MRSA), enterohemorrhagic *E. coli* O157:H7, *Pseudomonas*

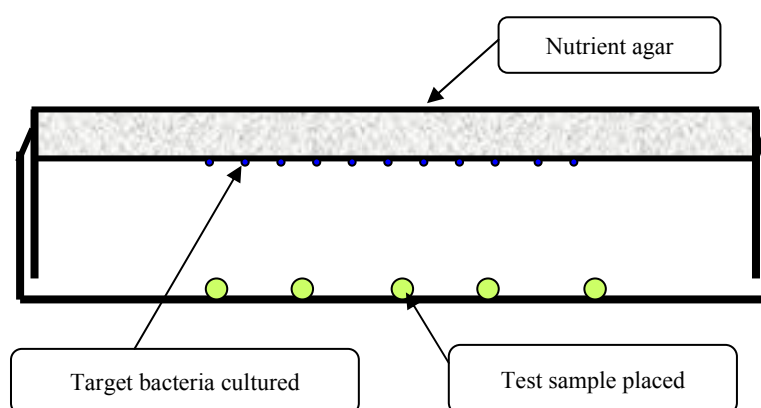


Fig. 2 Experimental illustration for antibacterial effect test of lavender flavor (volatile).

aeruginosa and *Candida albicans*, which were isolated from patients in hospital, except *E. coli*. All bacteria are clinically important at the medical field as the causative agents of human diseases, such as a food poisoning and hospital-acquired infectious diseases of the compromised hosts.

2.4 Antibacterial Test of Lavender Essential Oil and Oil-Emitted Flavor (Volatile)

Antibacterial potency of lavender oil was tested using the following criteria: (1) liquid medium cultivation analyses, using brain heart infusion (BHI) medium (Eiken Co. Ltd., Japan) by measuring turbidity of liquid medium at 655 nm; (2) BHI agar plate method, to observe whether colonies grow by or not by the manner as illustrated in Fig. 2 [7]. Briefly, the test sample of the extracted lavender oil was placed on a lid in petri dish, and the bacteria smeared on the agar plate was set upside down on lid of petri dish, followed by sealing the lid with an adhesive tape. The antibacterial effect of flavor was assessed by bacteria growth on the agar plate after overnight cultivation at 37 °C. Citronellal (commercial product, Tokyo Kasei Co. Ltd., Japan), one of the components of *Rosa rugosa*'s oil, was used as a positive control to compare antibacterial activity with that of the lavender's flavor.

3. Results

3.1 Chemical Constituents of Lavender Essential Oil

The hydrodistillation extracted 11.6 g of essential

oil from 1,168 g of lavender petals at a yield of 0.99% (w/w). Principal 10 components in essential oil accounted for 90% and were listed in Table 1. Total number of ingredients in lavender oil was 66 (Table 1).

Linalyl acetate, terpinen-4-ol and linalol were top three major compounds and occupied at 55% in the total quantity of lavender oil.

3.2 Assessment of Antibacterial Effect of Lavender Oil by Growth Curve in Liquid Medium

Lavender oil demonstrated an inhibitory activity against bacteria growth of *E. coli*, MRSA, O157:H7 and *C. albicans*, but *P. aeruginosa* resisted it and grew in the oil-contained liquid medium (BHI). Antibacterial act of citronellal was almost similar to that of lavender oil. Individual result obtained was demonstrated one by one in Figs. 3-7.

Table 1 Major compounds detected in the extracted lavender oil.

Compound	Amount (%)
Linalyl acetate	25.3
Terpinen-4-ol	16.4
Ocimene	3.6
Linalol	13
Lavandul acetate	7.1
Caryophyllene	6.5
β -farnesene	3.5
β -myrcene	2.5
1-octen-3-yl-acetate	1.7
Granyl acetate	1.7

Rest of elements detected were 10% in total amount.

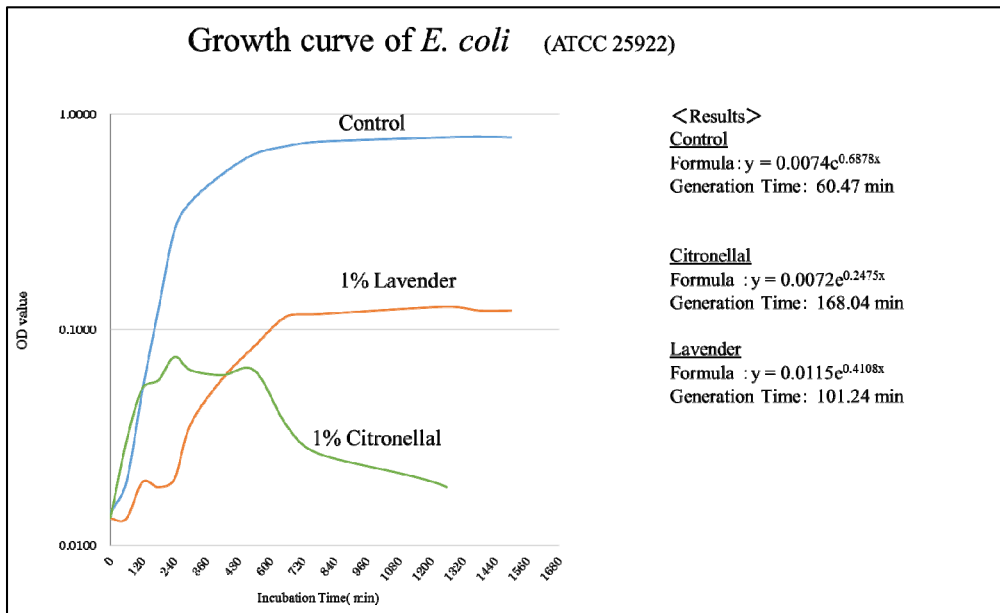


Fig. 3 Growth curve of *E. coli* in the lavender oil contained in BHI liquid medium.

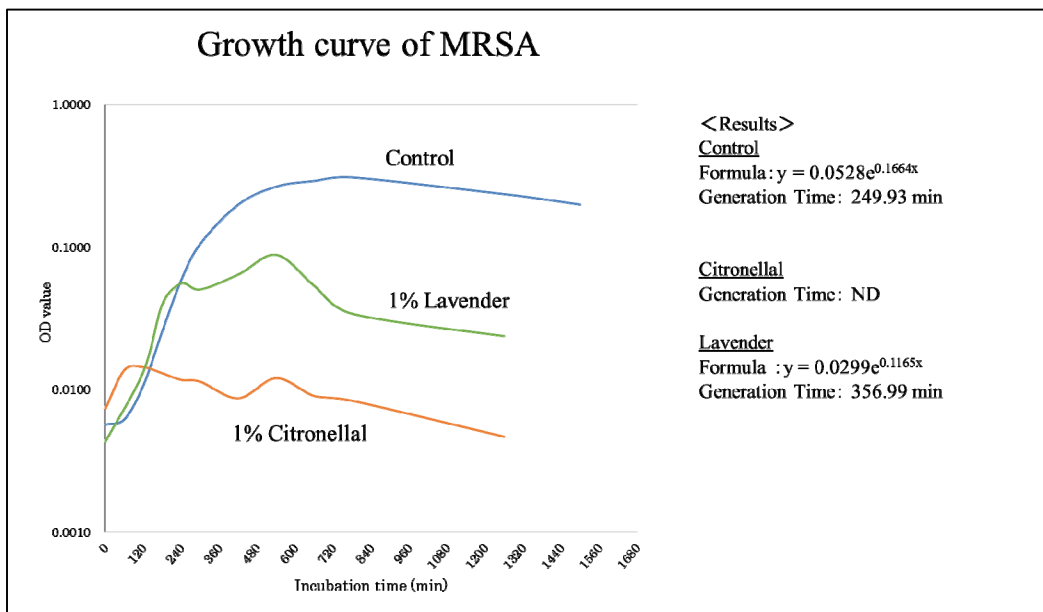


Fig. 4 Growth curve of MRSA in the lavender oil contained in BHI liquid medium.

In Fig. 3, it could be found that lavender oil worked efficiently against *E. coli* by inhibiting cell growth at log phase (2 h incubation time), thereafter cell death (lysis) was gradually induced (bottom line).

MRSA was resisted to the lavender oil, cell number was increasing at the log phase, and thereafter changed to decrease as following by incubation (middle line) (Fig. 4).

Fig. 5 showed that lavender oil worked to retard

proliferation of bacteria O157:H7 at early stage of log phase, followed by decreasing cells number (bottom line). Lavender oil seems to work as bacteria growth static by not allowing cell proliferation. Citronellal was less effective against bacteria growth.

Neither lavender nor citronellal oil was effective against *P. aeruginosa*. Bacteria continued growing in the oil present medium (Fig. 6).

Lavender oil appeared to work at early stage of lag

phase to prolong cell division. Thereafter, it allowed cell proliferation and reached at the same level of control after 44 h incubation. Citronellal was more effective in growth inhibition activity, and did not allow cell proliferation (bottom line) (Fig. 7).

Both lavender and citronellal oil worked to bacteria by inhibiting cell proliferation in the oil included medium. However, *P. aeruginosa* was exceptional and strongly resisted to the oil treatment. Working

manners (stage of growth curve) by the essential oil were different depending on the species of bacteria used.

3.3 Assessment of Antibacterial Effect of Lavender and Citronellal Flavor (Volatile) by Agar Plate Cultivation

Lavender- or citronellal-emitted flavor (volatile) demonstrated bacteria killing action as shown in Figs.

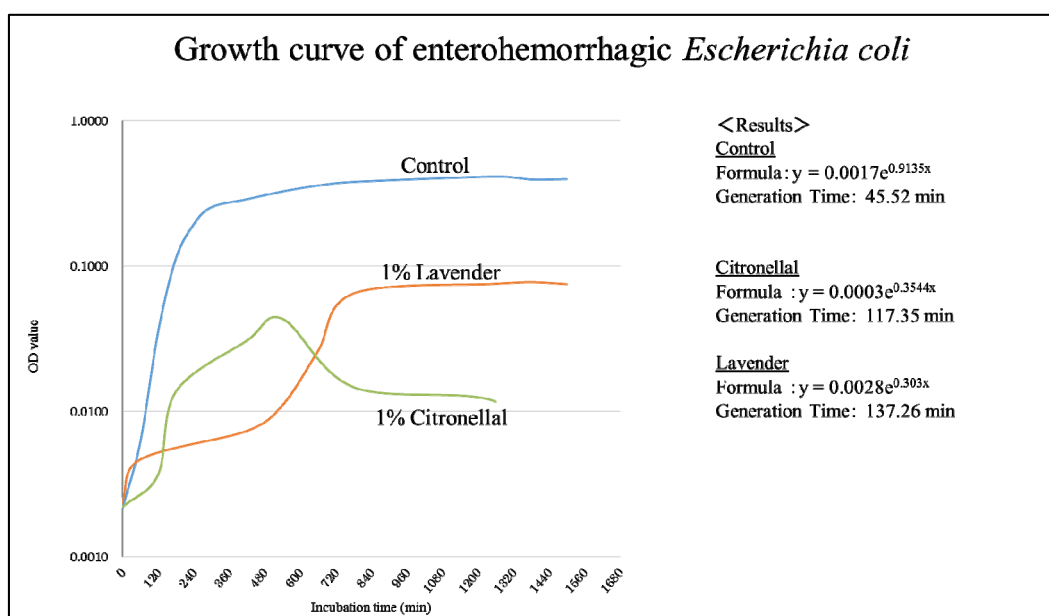


Fig. 5 Growth curve of enterohemorrhagi *E. coli* in the lavender oil contained in BHI liquid medium.

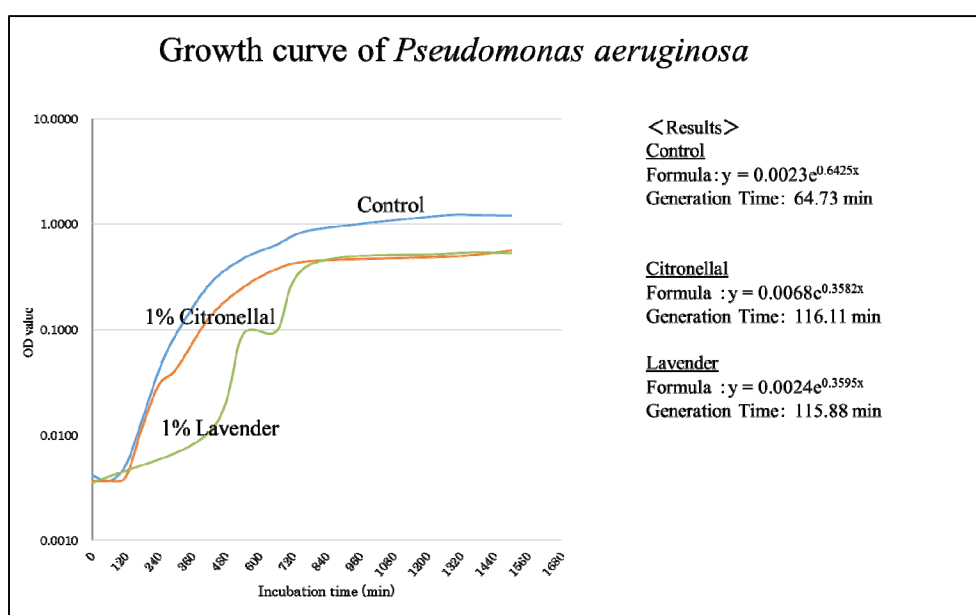


Fig. 6 Growth curve of *P. aeruginosa* in the lavender oil contained in BHI liquid medium.

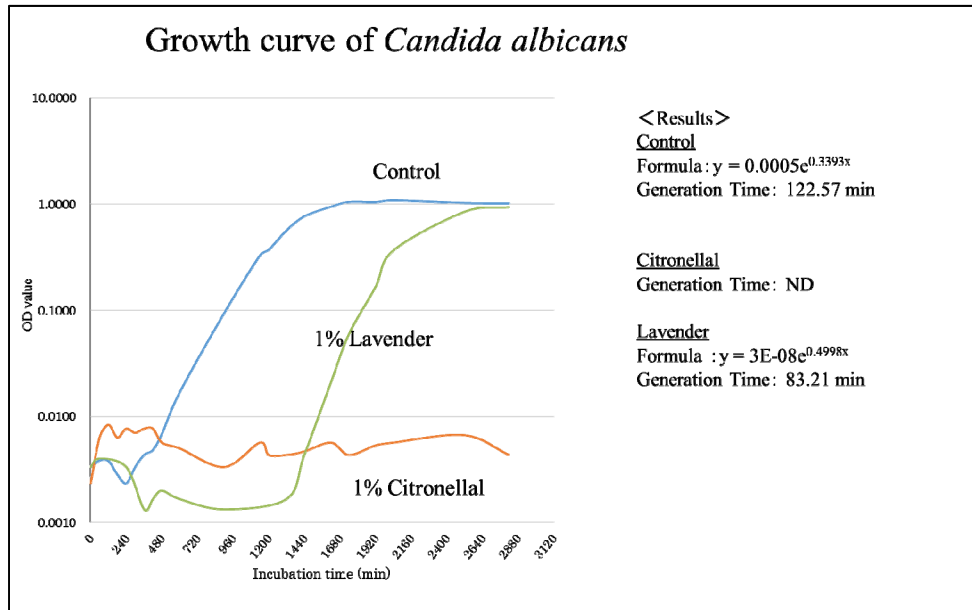


Fig. 7 Growth curve of *C. albicans* in the lavender oil contained in BHI liquid medium.

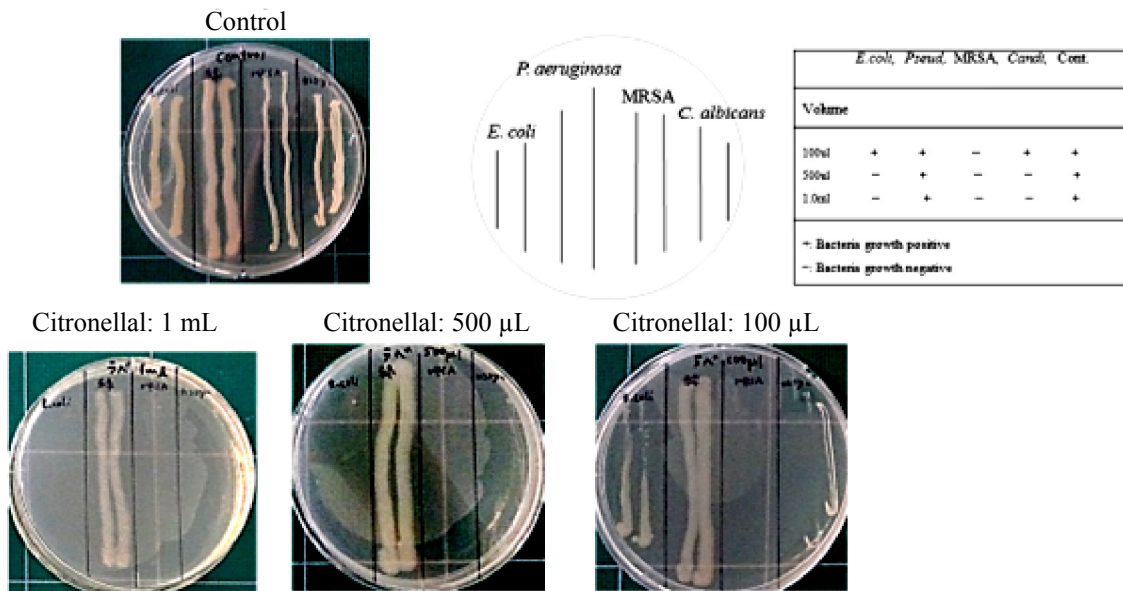


Fig. 8 Bacteria growth inhibition by the lavender emitted-flavor (volatile).

The table in top right part was enlarged and replaced in Table 2 to compare with that of citronellal.

8 and 9, and these results were summarized in Tables 2 and 3.

Fig. 8 showed that the growth inhibitive potency of lavender flavor (volatile) was dose dependent, and the flavor (volatile) released from 500 uL lavender oil completely blocked the growth of *E. coli*, MRSA and *C. albicans*. *P. aeruginosa* resisted to grow under this condition (Table 2).

The citronellal flavor (volatile) was less activity in

growth inhibition than that of the lavender. *E. coli*, O157 and *P. aeruginosa* grew under 500 uL concentration of citronellal flavor (Fig. 9). Results were listed in Tables 2 and 3 for the comparison of two compounds tested. The lavender and citronellal flavor demonstrated almost similar efficacy against bacteria used. Lavender flavor was more effective in the bacteria growth inhibition to compare with that of citronella.

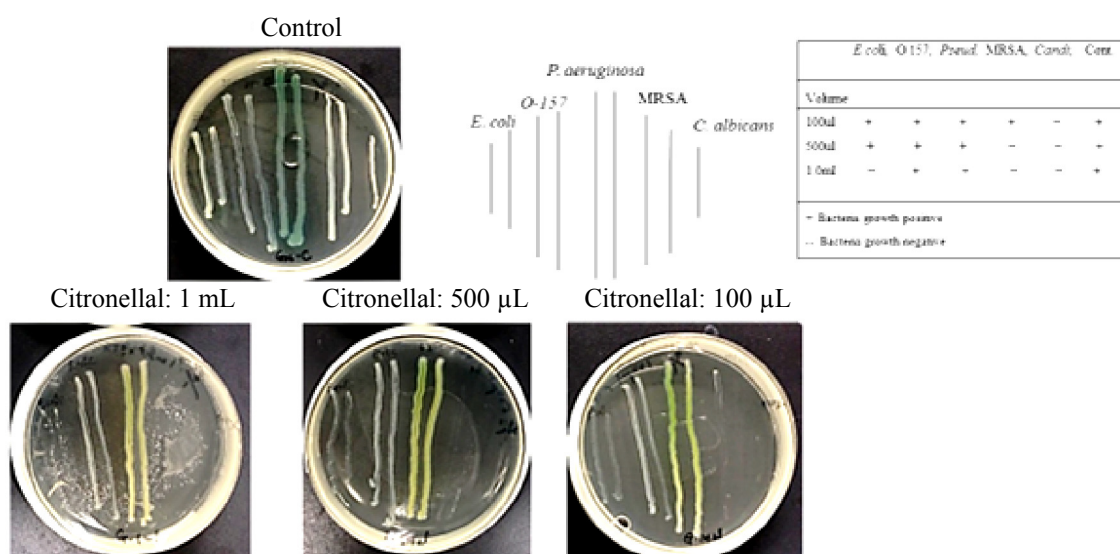


Fig. 9 Bacteria growth inhibition of the citronellal emitted-flavor (volatile).

The table in top right part was enlarged and replaced in Table 3 to compare with those of lavender.

Table 2 Comparative results of anti-bacteria potency of the lavender flavor (volatile).

Conc.	Lavender volatile			
	<i>E. coli</i>	<i>P. aeruginosa</i>	MRSA	<i>C. albicans</i>
100 uL	+	+	-	P+
500 uL	-	+	-	-
1.0 mL	-	+	-	-

+: growth positive; -: growth negative; P: partial growth.

Table 3 Comparative results of anti-bacteria potency of the citronellal flavor (volatile).

Conc.	Citronella volatile			
	<i>E. coli</i>	<i>P. aeruginosa</i>	MRSA	<i>C. albicans</i>
100 uL	+	+	+	P+
500 uL	+	+	-	-
1.0 mL	-	+	-	-

+: growth positive; -: growth negative; P: partial growth.

4. Discussion

Previously the data had demonstrated the antibacterial effect of the plants (vegetable)-emitted volatiles released from garlic, onion, horseradish, sage, whole clove, cinnamon, etc. [8]. Bacteria killing manners of these agents seemed different depending on the types of flavor released. The Hiba tree's (*Thujopsis dolabrata*) flavor-treated bacteria, for instance, showed a chapped surface with bleb creation from cell walls,

whereas the formalin gas-treated bacteria became flat in the shape without any blebs from cells by the scanning electron microscopic analyses.

Since it became clear in the present study that the lavender and citronellal flavor (oil) also possessed bacteria killing potency, this activity will be generalized in the field of plant flavor (volatile). This phenomenon might be considered to be the self-defense system of the plants to sustain their lives by fighting undesirable environmental agents, such as insect, bacteria and herbivore preference.

Plant-originated essential oils (flavor) are a rich source of bioactive phytochemicals and have been traditionally used for mental relaxation together with digestive complaints, such as loss of appetite, vomiting, upset stomach, migraine-headaches, toothaches, and to repel mosquitoes and other insects. In wide-ranging dietary, phytochemicals are found in fruits, vegetables, whole grains, nuts, seeds, fungi, herbs and spices, and have roles in the protection of human health, when their dietary intake is significant [9]. Therefore, beneficial application of the lavender oil (flavor) will be much expected in the medicinal field, such as the mental relaxation of the patient. Actually, a certain dentist in Japan has been using the

Hiba tree oil to sanitize (relax) the patient treatment room under relaxation of the patients. It is a kind of the forest bathing therapy in the clinic.

Phytochemicals are known to have biological properties to improve the human health, such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decreasing platelet aggregation and anticancer property [10]. However, less data have been presented until now on the antimicrobial action of the essential oil-emitted flavor (volatile).

It is urgent to accumulate more scientific data for safe application in the society. Because the potentially harmful effect of foods and flavorings was lately issued on experiment using DNA of cells from the laboratory study. They found that some foods activated cancer-linked gene p53, implying that the higher the level of DNA damage is, the more p53 becomes activated. However, they positively concluded that their studies do not suggest that people should stop using these foods, but do suggest the need for further research [11].

5. Conclusions

Antibacterial potency of the lavender-originated flavor (volatile) was tested against *E. coli*, MRSA, *P. aeruginosa* and *C. albicans* by using agar plate methods. The lavender or citronellal flavor (volatile) from *Rosa rugosa* demonstrated antibacterial potency, but *P. aeruginosa* was resistant to the flavor and could grow. The lavender oil availability in the society will be expected especially in the patient waiting room of the clinic.

Acknowledgments

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