Antibacterial Activity of the Mixed Systems Containing 1,2-Dodecanediol against *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Abstract: 1,2-Alkanediols are characteristic cosmetic ingredients because these moisturizers exhibit the antibacterial activity against *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*). However, the antimicrobial behavior in mixed systems containing several active ingredients is unclear because previous reports focus on an antibacterial system containing only 1,2-alkanediol. In this study, the minimal inhibitory concentration (MIC) and the fractional inhibitory concentration (FIC) were evaluated for 1,2-dodecanediol/lactic acid, 1,2-dodecanediol/myristic acid, 1,2-dodecanediol/methylparaben, and 1,2-dodecanediol/isopropyl methylphenol mixed systems to show the effect of the addition of other antimicrobial components to 1,2-dodecanediol. The antibacterial property of 1,2-dodecanediol/lactic acid mixed system was almost similar compared to 1,2-dodecanediol monomeric system. On the other hand, the antimicrobial activity of 1,2-dodecanediol against *S. epidermidis* was inhibited in the 1,2-dodecanediol/myristic acid mixed system. Because the selective antimicrobial activity of myristic acid against *S. aureus* was demonstrated in the mixed system. The present findings are useful for designing formulations of cosmetics and body cleansers containing 1,2-dodecanediol.

Key words: antibacterial activity, combination effect, *Staphylococcus*, minimal inhibitory concentration, fractional inhibitory concentration

1 Introduction

1,2-Alkanediols exert a moisturizing effect and exhibit antibacterial activity against *Escherichia coli* and *Aspergillus niger* [5-7]. In a previous study, we evaluated the antimicrobial activity of 1,2-alkanediols against *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) and found that the antimicrobial activity of these surfactants was stronger against both *Staphylococci*, if the alkyl chain length was longer [8]. We selected these bacteria, as *S. aureus* causes atopic dermatitis and skin roughness, whereas *S. epidermidis* enhances innate immunity and maintains healthy epidermis [7-10]. Lee et al. reported that 1,2-alkanediols exhibit antibacterial properties as the results of the disruption of the cell membrane [11]. We also considered that 1,2-dodecanediol can penetrate the cell membrane and inhibit the growth of *S. aureus* and *S. epidermidis* owing to its hydrophobic long alkyl chain.

The antimicrobial behavior of 1,2-alkanediol in mixed systems with other components is unclear as only a single antimicrobial agent was added in these evaluation systems in previous study. However, various antimicrobial ingredients such as acids, salts, sebum and preservatives can coexist on the surface of the skin, if cosmetic and cleansing products containing 1,2-alkanediol are applied to keep the body clean and healthy [12-14]. These substances may affect the antimicrobial properties against skin bacteria. The antibacterial ability against *S. aureus* or *E. coli* was improved when 1,2-alkanediol was mixed with dipropylene glycol or phenoxyethanol [15, 16]. Therefore, the antimicrobial behavior in mixed systems, including multiple antimicrobial agents, is important for the preparation of effective antimicrobial systems. The checkerboard method is useful for evaluating
the antimicrobial activity of systems containing multiple ingredients. The synergistic or antagonistic effects of two antimicrobial agents can be determined by calculating the fractional inhibitory concentration (FIC) index based on the growth inhibition effect of the combination of the two agents^{17,19}. The activity of antibiotic combinations, such as imipenem and vancomycin, against methicillin-resistant bacteria has been evaluated using this method^{19-22}.

In the present study, the checkerboard method was used to evaluate the antimicrobial activity of a mixture of 1,2-dodecanediol and other antimicrobial substances against S. aureus and S. epidermidis. Lactic acid and myristic acid, which are antimicrobial substances on the surface of the skin, and methylparaben and isopropyl methylphenol, which are typical preservatives and bactericidal ingredients in cosmetics, were selected as additional ingredients^{23-26}. The antibacterial activity of these substances was determined using minimal inhibitory concentration (MIC), which is the minimum drug concentration required to suppress bacterial growth, and the combined effect of the two-component mixture was evaluated based on the FIC index. The findings of the present study are useful for designing skin care and cleansing products focusing on human skin flora.

2 Experimental
2.1 Materials

The molecular structures of the materials used in this study are shown in Fig. 1. 1,2-Dodecanediol (98%) and myristic acid (C14:0 fatty acid, ≥ 99%) were purchased from Sigma-Aldrich Co. LLC (St. Louis, USA). Lactic acid, methyl paraben (≥ 99%), 4-isopropyl-3-methylphenol (≥ 99%) and 0.1 mol L⁻¹ phosphate buffer solution (pH 6) were obtained from FUJIFILM Wako Pure Chemical Industries Ltd. (Osaka, Japan). Ethanol was purchased from Junsei Chemical Co. (Tokyo, Japan). Sodium chloride was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Beef extract was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Polypeptone was purchased from Nihon Pharmaceutical Co., Ltd. (Tokyo, Japan). S. aureus (NBRC13276) and S. epidermidis (NBRC12993) were obtained from the National Institute of Technology and Evaluation (Tokyo, Japan). Water was purified using a Demi-Ace Model DX-15 demineralizer (Kurita Water Industries Ltd., Tokyo, Japan).

2.2 Preparation of culture medium and bacterial dispersion

Before the cultivation of S. aureus and S. epidermidis, a medium containing beef extract (0.15 g), polypeptone (0.30 g), sodium chloride (0.15 g), and water (30 mL) was sterilized in an autoclave at 121°C for 20 min. The S. aureus and S. epidermidis cultures were prepared by shaking 30 mL of the medium containing one colony for 24 h at 37°C (145 rpm). For the MIC evaluation and the assays using the checkerboard method, the medium containing beef extract (1.50 g), polypeptone (3.00 g), sodium chloride (1.50 g), and pH 6 phosphate buffer (150 mL) were sterilized in an autoclave at 121°C for 20 min.

2.3 Evaluation of MIC against S. aureus and S. epidermidis

The MIC was defined as the minimum concentration at which turbidity did not increase when the antibacterial agents were added to the bacterial dispersion. First, lactic acid was diluted with phosphate buffer, while 1,2-dodecanediol, myristic acid, methylparaben, and isopropyl methylphenol were diluted with ethanol to prepare the following sample solutions: 3.13 ~ 400 µg mL⁻¹ (1,2-dodecanediol), 300 ~ 32,000 µg mL⁻¹ (lactic acid), 100 ~ 2500 µg mL⁻¹ (myristic acid), 21.9 ~ 3200 µg mL⁻¹ (methylparaben), and 9.4 ~ 1000 µg mL⁻¹ (4-isopropyl-3-methylphenol). We confirmed that the addition of 2 wt% ethanol did not affect the antibacterial properties in preliminary tests. Next, 1000

![Fig. 1](image_url) Molecular structure of (a) 1,2-dodecanediol, (b) myristic acid, (c) lactic acid, (d) methylparaben, and (e) 4-isopropyl-3-methylphenol.
µL, a liquid medium containing beef extract (0.01 g), polypeptide (0.02 g), and sodium chloride (0.01 g), 940 µL phosphate buffer (pH 6), and 20 µL bacterial dispersion (3 × 10^5–2 × 10^6 CFU mL⁻¹) were added to 40 µL ethanol solution of antimicrobial agents.

The optical density (OD₆₆₀) of the prepared sample (100 µL) was evaluated using a 96-well TrueLine Cell Culture Plate (Japan Genetics Co., Ltd., Tokyo, Japan) and a microplate reader SH-1200 Lab (Corona Electric Co., Ltd., Kyoto, Japan). The evaluated conditions were as follows: wavelength, 660 nm; number of flashes, 10. The S. aureus medium was shaken at 1000 rpm by a microplate shaker PSU-2T (Waken B Tech Co., Ltd., Kyoto, Japan) during incubation for 24 h at 37°C. On the other hand, the dispersion containing S. epidermidis was shaken for 48 h under the same conditions because the growth rate of S. epidermidis is slower compared with that of S. aureus.

2.4 Evaluation of the combined effect of antibacterial components based on checkerboard method

The effects of lactic acid, myristic acid, methylparaben, and isopropyl methylphenol on the antibacterial properties of 1,2-dodecanediol were evaluated using the checkerboard method, a typical evaluation method for mixed antibacterial systems. In general, a combined effect is determined based on the FIC index, which is calculated from the MIC of a mixture containing two types of drugs. Here, the antimicrobial activity of the mixtures containing 1,2-dodecanediol and the additives in the concentrations of 1/64 to 4 times the MIC was evaluated: the concentration ranges of 1,2-dodecanediol and additives were as follows: 0.78–200 µg mL⁻¹ (1,2-dodecanediol; S. aureus, S. epidermidis), 3.55–16000 µg mL⁻¹ (lactic acid; S. aureus), 50.0–12800 µg mL⁻¹ (lactic acid; S. epidermidis), 2.45–628 µg mL⁻¹ (myristic acid; S. aureus), 6.25–1600 µg mL⁻¹ (myristic acid; S. epidermidis), 2.50–6400 µg mL⁻¹ (methylparaben; S. aureus, S. epidermidis), and 2.16–552 µg mL⁻¹ (isopropyl methylphenol; S. aureus), and 2.70–700 µg mL⁻¹ (isopropyl methylphenol; S. epidermidis).

To evaluate the combined effect, 20 µL of a 1,2-dodecanediol ethanol solution, 20 µL of ethanol solution of the additive, 1000 µL of liquid medium containing beef extract (0.01 g), polypeptide (0.02 g), and sodium chloride (0.01 g), 940 µL of phosphate buffer solution and 20 µL of a bacterial dispersion (3 × 10^5–2 × 10^6 CFU mL⁻¹) were mixed in a test tube. When lactic acid was added, 1000 µL of the liquid medium, 940 µL of phosphate buffer solution containing lactic acid and 20 µL of bacterial solution was added to 40 µL of 1,2-dodecanediol ethanol solution. Lactic acid was dispersed in a phosphate buffer solution at pH 6 because the antibacterial effect of organic acid is dependent on the pH concentration.

The prepared sample (100 µL) was added to a 96-well TrueLine cell culture plate. The plate diagram used for the evaluation of the 1,2-dodecanediol/lactic acid mixed system against S. aureus is shown in Fig. S1 (see supporting information). The OD₆₆₀ of these samples was evaluated by grating microplate reader (SH-1200 Lab). The conditions were as follows: wavelength, 660 nm; number of flashes, 10. Samples containing S. aureus or S. epidermidis were shaken at 1000 rpm by PSU-2T and were incubated at 37°C for 24 and 48 h, respectively. To confirm the reproducibility, two pairs of plates containing the same composition were prepared and were evaluated twice. In order to calculate the FIC index and ΣFIC, the obtained turbidity was shown in a 9 × 9 checkerboard diagram. This turbidity is the difference between the turbidities at 0 h and 24 (or 48) h to exclude the effect of the precipitation of components. The FIC was calculated for each antimicrobial combination as the sum of the individual MICs. In the board diagram, the mean turbidity values are shown in five levels, OD₆₆₀ = 0.000, 0.150, 0.300, 0.450, 0.600, and 0.750.

2.5 Determination of the combination effect

The FIC index of the mixed system consisting of drugs A and B was calculated using the following equation:

\[ \text{FIC index} = \frac{\text{MIC of tested A in combination}}{\text{MIC of tested A only}} + \frac{\text{MIC of tested B in combination}}{\text{MIC of tested B only}} \]

Based on the ΣFIC values, the interactions between drugs A and B were categorized into the following: (1) synergistic; when ΣFIC ≤ 0.5, the effect of the two drugs combined with much stronger than the sum of the effects of the single drug, (2) additive; when 0.5 < ΣFIC ≤ 1.0, two drugs combined are slightly more effective than a single drug, (3) indifferent; when 1.0 < ΣFIC ≤ 2.0, the combined effect is neither improved nor inferior to that of the single agent, (4) antagonistic; when 2.0 < ΣFIC, the effect of the combination is inferior to the action of the single agent.

Figures S2(a) ~ (d) in supporting information are examples of board diagrams when the combined effects of drugs A and B are synergistic, additive, indifferent, or antagonistic, respectively. The horizontal and vertical axes of the board diagram indicate the concentration of drugs A and B, respectively. Turbidity was increased with bacterial growth under the colored conditions in the board diagram. In these examples, the ΣFIC is (a) 0.194, (b) 0.661, (c) 1.164, and (d) 3.10, respectively.

3 Results

3.1 MIC of 1,2-dodecanediol and additives against S. aureus and S. epidermidis

Liquid medium containing S. aureus or S. epidermidis was added to 1,2-dodecanediol, lactic acid, myristic acid, and isopropyl methylphenol in a phosphate buffer solution.
methylparaben, and isopropyl methylphenol solutions and incubated at 37°C for 24 or 48 h, respectively. The temporal change of the OD660 was as follows:

\[ \Delta \text{OD660} \]

(a) 1,2-dodecanediol. The antimicrobial activity of 1,2-dodecanediol against *S. aureus* or *S. epidermidis* was evaluated in a previous study. \(^6\) Figures 2(a) and (b) show the temporal changes in the OD660 of the liquid medium containing 1,2-dodecanediol and *S. aureus* or *S. epidermidis*. The white circles indicate the OD660 immediately after the addition of the bacteria to the liquid medium, and black ones are that at 24 or 48 h, respectively. In these profiles, the growth of the bacteria is inhibited if the turbidity does not increase. Here, the OD660 increased when the 1,2-dodecanediol concentration was less than 50.0 µg mL\(^{-1}\) for both *S. aureus* and *S. epidermidis*; the MIC of 1,2-dodecanediol was 50.0 µg mL\(^{-1}\) where bacterial growth was not observed against *S. aureus* and *S. epidermidis*.

(b) Lactic acid. Figures 3(a) and (b) show the temporal changes in the OD660 of the liquid medium containing lactic acid and *S. aureus* or *S. epidermidis*. Here, turbidity increased at less than 4000 µg mL\(^{-1}\) for *S. aureus* and less than 3200 µg mL\(^{-1}\) for *S. epidermidis*; the MICs against *S. aureus* and *S. epidermidis* were 4000 µg mL\(^{-1}\) and 3200 µg mL\(^{-1}\), respectively.

(c) Myristic acid. Figures 4(a) and (b) show the temporal changes in the OD660 of the liquid medium containing myristic acid and *S. aureus* or *S. epidermidis*. The turbidity increased with the increase of the concentration at 0 h, because insoluble myristic acid was dispersed in the medium. In the case of *S. aureus*, turbidity increased at myristic acid concentrations less than 180 µg mL\(^{-1}\), whereas for *S. epidermidis*, turbidity increased at all fatty acid concentrations. Furthermore, the MICs against *S. aureus* were 140, 150, and 180 µg mL\(^{-1}\) in the three times antimicrobial tests. Therefore, the MIC of myristic acid against *S. aureus* was set to 157 µg mL\(^{-1}\) in this study, which is the average of the obtained MICs. Significant antimicrobial activity was not observed against *S. epidermidis*; the MIC was above 2500 µg mL\(^{-1}\).
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Fig. 4  Inhibitory effects of myristic acid on bacterial growth. (a) S. aureus, (b) S. epidermidis (○: 0 h, ●: 24(48) h).

(d) Methylparaben. Figures 5(a) and (b) show the temporal changes in the OD_{660} of the liquid medium containing methylparaben and S. aureus or S. epidermidis. The OD_{660} increased when the concentration was less than 1600 µg mL^{-1} for both S. aureus and S. epidermidis: the MICs were 1600 µg mL^{-1} against both S. aureus and S. epidermidis.

(e) Isopropyl methylphenol. Figures 6(a) and (b) show the temporal changes in the OD_{660} of the liquid medium containing isopropyl methylphenol and S. aureus or S. epidermidis. Here, turbidity increased at less than 150 µg mL^{-1} for S. aureus and less than 200 µg mL^{-1} for S. epidermidis. The MIC against S. aureus varied from 125 to 150 µg mL^{-1} in the three times antimicrobial tests. Similarly, the MIC against S. epidermidis varied from 150 to 200 µg mL^{-1}. Therefore, the MIC of isopropyl methylphenol against S. aureus and S. epidermidis were set to 138 and 175 µg mL^{-1}, respectively, which were the averages of the obtained MICs.

3.2 Evaluation of the combination effect

The antimicrobial activity was evaluated for a mixed system containing 1,2-dodecanediol and four additives, lactic acid, myristic acid, methylparaben, and isopropyl methylphenol against S. aureus and S. epidermidis. Culture medium containing S. aureus or S. epidermidis was added to a mixture of 1,2-dodecanediol and to each substance, respectively. The OD_{660} was measured after incubation at 37°C for 24 or 48 h.

(a) 1,2-dodecanediol/lactic acid mixed system. Board diagrams of the antibacterial activity of 1,2-dodecanediol/lactic acid mixed system against S. aureus and S. epidermidis are shown in Figs. 7(a) and (b), respectively. In the case of 1,2-dodecanediol/lactic acid mixture, the turbidity did not increase when concentrations of 1,2-dodecanediol and lactic acid were above 50.0 and 4000 µg mL^{-1}, respectively. These concentrations were consistent with the MICs of 1,2-dodecanediol and lactic acid individually. The ΣFIC for this mixed system was 1.29 ± 0.09, which suggests that the antibacterial behavior of 1,2-dodecanediol and lactic acid against S. aureus was “indifferent”.

The antimicrobial behavior of the 1,2-dodecanediol/lactic acid mixed system against S. epidermidis was similar to
that against *S. aureus*. Antimicrobial activity was confirmed as the turbidity did not increase when the concentration of 1,2-dodecanediol and lactic acid was above the respective MICs of 50.0 and 3200 µg mL⁻¹. In the case of this mixed system, antimicrobial behavior was "indifferent" against *S. epidermidis* because ΣFIC was 1.23 ± 0.11.

(b) 1,2-dodecanediol/myristic acid mixed system. Figures 8(a) and (b) show the board diagram of the antibacterial activity of 1,2-dodecanediol/myristic acid mixed system against *S. aureus* and *S. epidermidis*, respectively. The 1,2-dodecanediol/myristic acid mixture exhibited an antibacterial against *S. aureus* as the turbidity did not increase when concentrations of 1,2-dodecanediol and myristic acid were above 50.0 and 78.5 µg mL⁻¹, respectively. These results suggest that the antimicrobial behavior of this mixed system is "additive" as ΣFIC of mixed system was 0.89 ± 0.24.

On the other hand, the antimicrobial behavior of this binary mixture against *S. epidermidis* was complicated. In this mixed system, the turbidity of the liquid medium increased even when it included 50.0 µg mL⁻¹ of 1,2-dodecanediol. This suggests that myristic acid inhibited the antibacterial activity of 1,2-dodecanediol. The extent of this inhibition intensified when the amount of myristic acid was increased. When the concentration of myristic acid was 6.25 µg mL⁻¹, the antibacterial phenomenon was confirmed under the condition of more than 100 µg mL⁻¹ of 1,2-dodecanediol. However, the turbidity increased even with the addition of 100 µg mL⁻¹ of 1,2-dodecanediol when the concentration of myristic acid was 200 µg mL⁻¹. On the other hand, in the system containing 0.78 µg mL⁻¹ of 1,2-dodecanediol and 1600 µg mL⁻¹ of myristic acid, turbidity increased from 0.164 to 0.277 in 48 h. This board diagram suggests that this increase is caused by the growth of bacteria. Therefore, the mixture containing myristic acid did not show antimicrobial activity against *S. epidermidis*.
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(c) 1,2-dodecanediol/methylparaben mixed system. Figures 9(a) and (b) show the board diagram of the antibacterial activity of 1,2-dodecanediol/methylparaben mixed system against *S. aureus* and *S. epidermidis*, respectively. The 1,2-dodecanediol/methylparaben mixture exhibited an antibacterial activity against *S. aureus* as the turbidity did not increase when the concentrations of 1,2-dodecanediol and methylparaben were above 100 and 1600 µg mL⁻¹, respectively. \( \Sigma \text{FIC} \) was 1.38 ± 0.16, indicating that the antimicrobial properties of methylparaben and 1,2-dodecanediol are indifferent against *S. aureus*.

On the other hand, turbidity did not increase against *S. epidermidis* when the concentrations of 1,2-dodecanediol and methylparaben were 100 and 3200 µg mL⁻¹, respectively. The MICs of these components are 50.0 and 1600 µg mL⁻¹, respectively. It was confirmed that the coexistence of the two components required the addition of a large amount of antimicrobial components. The antimicrobial behavior of 1,2-dodecanediol and methylparaben was “antagonistic” because \( \Sigma \text{FIC} \) for this binary system was 2.14 ± 0.20.

(d) 1,2-dodecanediol/isopropyl methylphenol mixed system. Figures 10(a) and (b) show the board diagram of the antibacterial activity in 1,2-dodecanediol/isopropyl methylphenol mixed system against *S. aureus* and *S. epidermidis*, respectively. In this mixture, the turbidity did not increase against *S. aureus* when concentrations of
1,2-dodecanediol and isopropyl methylphenol were above 50.0 and 276 µg mL⁻¹, respectively. The ΣFIC was 1.34 ± 0.16, which suggests that the antibacterial activities of 1,2-dodecanediol and isopropyl methylphenol against *S. aureus* were "indifferent".

The antimicrobial behavior of the 1,2-dodecanediol/isopropyl methylphenol mixed against *S. epidermidis* was similar to that against *S. aureus*. Antimicrobial activity was confirmed as the turbidity did not increase when the concentrations of 1,2-dodecanediol and isopropyl methylphenol were above 50.0 and 350 µg mL⁻¹, respectively. The behavior of this mixed system was "indifferent" against *S. epidermidis* as ΣFIC was 1.29 ± 0.30.

### 4 Discussion

As shown in Table 1, four additives showed additive, indifferent, or antagonistic effects to 1,2-dodecanediol. These characteristic behaviors reflected the antibacterial mechanism of each substance. In the case of 1,2-dodecanediol/lactic acid mixed system, no synergistic effect was observed in the antimicrobial activity against both *Staphylococci*. In particular, the turbidity did not increase when the concentration of lactic acid was above the MIC. This was caused by a decrease in the intracellular pH of the bacteria due to the addition of lactic acid. Some previous studies showed that a reduction of pH inside the bacteria cell is a mainly mechanism of the antibacterial effect of acidic materials. At a low pH condition, undissociated acids can permeate the cell membrane and flow into the intracellular regions. In general, hydrogen ion H⁺ is dissociated from acidic molecules because the pH of the cytoplasm is higher compared with the environment external to the bacteria. This causes the inhibition of growth of the bacteria because the pH range is beyond the optimal range for biological reactions. The pH of 1,2-dodecanediol/lactic acid mixed system was 3.2–4.4 when the concentration of lactic acid was above 4000 µg mL⁻¹, which is the MIC against *S. aureus*. The pH of these mixed systems was out of the buffer region of the phosphate buffer (pH 6), because they contained high concentration of lactic acid. Since the pKa value of lactic acid is 3.83, a system containing more than 4000 µg mL⁻¹ lactic acid would be expected to have many lactic acid molecules in an undissociated form.

### Table 1

| Strains        | Drug combination         | ΣFIC index | Interpretation    |
|----------------|--------------------------|------------|-------------------|
| *S. aureus*    | 1,2-dodecanediol+Lactic acid | 1.29±0.09  | indifference      |
|                | 1,2-dodecanediol+Myristic acid | 0.89±0.24  | additive          |
| NBRC13276      | 1,2-dodecanediol+Methyl paraben  | 1.38±0.16  | indifference      |
|                | 1,2-dodecanediol+Isopropylmethylphenol | 1.34±0.16  | indifference      |
| *S. epidermidis*| 1,2-dodecanediol+Lactic acid | 1.23±0.11  | indifference      |
| NBRC12993      | 1,2-dodecanediol+Myristic acid | —          | —                 |
|                | 1,2-dodecanediol+Methyl paraben  | 2.14±0.20  | antagonism        |
|                | 1,2-dodecanediol+Isopropylmethylphenol | 1.29±0.30  | indifference      |
1,2-dodecanediol/myristic acid mixed system showed a slight stronger selective antibacterial activity, because the antimicrobial effects of both surfactants are due to their effects on cell membranes. Desbois et al. reported that fatty acids affect the membrane function through the solubilization of cell membranes, disruption of electron transport chains, inhibition of oxidative phosphorylation, impaired enzyme activity, and formation of autooxidative degradation products\(^\text{34–36}\). On the other hand, an increase in turbidity was observed for \textit{S. epidermidis} regardless of the mixture of 1,2-dodecanediol and myristic acid. This reflects the selective antibacterial properties of myristic acid: Myristic acid shows antibacterial activity against \textit{S. aureus}, but not against \textit{S. epidermidis}\(^7\). That is, the fact that antimicrobial activity against \textit{S. epidermidis} was not observed under many conditions indicated that the selective antimicrobial activity of myristic acid was maintained even in a two-component mixture. Conversely, the antimicrobial activity of 1,2-dodecanediol against \textit{S. epidermidis} was inhibited. As shown in Fig. 8(a), if 50.0 \(\mu\text{g mL}^{-1}\) of 1,2-dodecanediol, which is the MIC, was added, the antibacterial activity against \textit{S. epidermidis} was not observed. The exact mechanism of this antimicrobial behavior is unclear.

In the case of 1,2-dodecanediol/methylparaben mixed system, the antimicrobial behavior was indifferent against \textit{S. aureus} and antagonistic against \textit{S. epidermidis}. 1,2-dodecanediol/isopropyl methylphenol mixed system showed that synergistic effect was not found against both \textit{Staphylococci}. It is difficult to understand the exact antibacterial mechanism of these mixed systems. Phenolic compounds such as methylparaben and isopropyl methylphenol are often thought to exhibit antimicrobial properties by damaging cell membranes and causing changes in the membrane function and structure\(^38–40\). However, these mechanisms are not clear. In this study, the antagonism of 1,2-dodecanediol and methylparaben against \textit{S. epidermidis} may not only be due to the antimicrobial mechanism, but also to a complicated action, for example, the regulation of bacterial gene transcription in response to change in the external environment\(^41\).

When the concentration of amphiphilic molecules is more than the critical micelle concentration (CMC), the molecules form the self-assembling structure. In most of the cases in this study, antimicrobial activity was observed when the concentration of 1,2-dodecanediol was above MIC. 1,2-dodecanediol may form aggregates at MICs; the MIC against \textit{Staphylococci} and CMC of 1,2-dodecanediol are 50.0 \(\mu\text{g mL}^{-1}\) and 36.8 \(\mu\text{g mL}^{-1}\), respectively\(^42\). These results suggest that 1,2-dodecanediol, which is highly amphiphilic, may penetrate cell membranes and exhibit antimicrobial activity. Previously, some researchers reported that micelle formation affects the antibacterial property of antibacterial agents. For example, the antimicrobial activity of the phenol type preservative is weakened in micellar solution of nonionic surfactants, because the molecules are solubilized in the micelles or form a hydrophobic complex\(^43–46\). On the other hand, Tobe et al. reported that bactericidal activities of benzalkonium chloride is stronger when the concentration of methyl ester ethoxylates type nonionic surfactant is greater than the CMC\(^\text{47}\).

5 Conclusion
The antibacterial activity of the mixed system of lactic acid, myristic acid, methylparaben, and isopropyl methylphenol with 1,2-dodecanediol was evaluated against \textit{S. aureus} and \textit{S. epidermidis} using the checkerboard method. The antibacterial activities did not significantly change in the 1,2-dodecanediol/lactic acid from that each component monomeric system. In the 1,2-dodecanediol/myristic acid mixed system, the antibacterial activity against \textit{S. aureus} was slightly improved, and no clear was observed antimicrobial activity against \textit{S. epidermidis}. The antibacterial activity of 1,2-dodecanediol against \textit{S. epidermidis} was inhibited by myristic acid. On the other hand, the antimicrobial activities of mixed systems, including 1,2-dodecanediol and phenolic compounds, were complicated. The 1,2-dodecanediol/methylparaben mixed system showed that no variation in the antibacterial activity of each component against \textit{S. aureus}, but the two components tended to be antagonistic against \textit{S. epidermidis}. In the case of 1,2-dodecanediol/isopropyl methylphenol mixture, although FIC showed that the antimicrobial activity was indifferent to both \textit{Staphylococci}, the turbidity increased with the addition of MIC of isopropyl methylphenol under some conditions. In particular, methylparaben and isopropyl methylphenol are added to cosmetic products and body cleansers as preservative and sterilization agents. These results of this study predict that these antimicrobial properties may be altered when 1,2-dodecanediol is added to the product. These findings are useful for cosmetic and body cleansers formulation design.

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Supporting Information
This material is available free of charge via the Internet at https://dx.doi.org/jos.70.10.5650/jos.ess20362
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