Antibacterial and antifungal activities of isoprenoids

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We assayed biologically significant isoprenoids for antibacterial and antifungal activities. Isoprenoids varied widely with respect to their growth inhibitory activities towards Gram-positive and Gram-negative bacteria and fungal species. Of particular interest was the finding that geraniol acted as a broad spectrum of antibiotic, and significantly inhibited the growth of Gram-positive *Staphylococcus aureus* 209P and methicillin resistant *S. aureus* 834, Gram-negative *Escherichia coli* IFO-3806, and *Shigella sonnei*, as well as the fungus *Trichopyton* sp. Farnesol inhibited *S. aureus* strains 209P and 834 to a greater extent than did geraniol, but did not detectably inhibit Gram-negative bacteria.

Key words: Antibacterial assay, Gram-positive bacteria, Gram-negative bacteria, fungi, isoprenoids

1. INTRODUCTION

"Shinrin-yoku" is the Japanese term for spending time in forests in order to benefit from the stress-relieving and refreshing effects of an atmosphere filled with biological response modifiers produced by the vegetation. [1-5]. The substance which leads to these effects is called phytoncide. Phytoncides are defined as substances produced by all types of plants that have an influence on other organisms[2,6]. Phytoncides can defend one plant species from another [6,7], exert toxic effects on insects [6-10], and inhibit the growth of pathogenic bacteria [1-3, 6].

Among the volatile organic compounds termed "phytoncide", the isoprenoids which show biological activities, such as antimicrobial actions, are also contained.

Further, huge researches of antimicrobial agents have been done for many years. And developments of new antimicrobial agents whenever resistant bacteria appear have been repeated [11-19].

Based on the limited knowledge of isoprenoids' antimicrobial effects, we undertook studies aimed to determine whether further characterization could lead to useful new drugs. We were able to take advantage of our isoprenoid collection developed in our studies of prenyltransferases [20-22]. We performed the antimicrobial activity examination using some synthesized isoprenoids and some marketed commodities.

This paper describes the antimicrobial properties of select isoprenoids when tested on

Gram-positive and Gram-negative bacterial and fungal species.

2. EXPERIMENTAL

2.1 Agar plate dilution method

Antibacterial assays were performed in accordance with the agar plate dilution method recommended by the Japanese Society of Chemotherapy [23-25].

Bacteria were inoculated into 10 ml of Mueller-Hinton Broth medium (MHB, Difco™, Becton Dickinson (BD), USA) and cultured at 37°C for 24 h without shaking. Each isoprenoid was dissolved in DMSO or a 5% aqueous solution of the surfactant HCO-50 (w/v) (Nikko Chemicals Company Ltd., Japan), and 120 µL were added to 12 ml Mueller-Hinton Agar (MHA) culture medium.

The optical density (OD) of bacterial cultures at 550 nm was determined using a Smart Spec™ 3000 (Bio-Rad Laboratories, USA) prior to performing colony counts.

Cultures were adjusted to 1×10^6 colony-forming units (CFU)/ml, and colonies were counted after incubation at 37 °C for 20 h. Fungi were cultured in Sabouraud Dextrose agar medium (Eiken Chemical Co., Ltd., Japan) at 25-27 °C

The Minimal Inhibitory Concentration (MIC) at which a molecule exerted antimicrobial activity was defined as less than 800 µg/mL in accordance with the criterion set forth by the Japanese Society of Chemotherapy [23-25]. Media were sterilized using an STH 364 FA autoclave

(Advantec Toyo Kaisha, Ltd.).

2.2 Bacterial strains

Gram-positive bacteria used were the following: *S. aureus* 209P, *S. aureus* 834 (methicillin resistant - MRSA) and *Listeria monocytogenes* 1b-1684; and Gram-negatives strains were *E. coli* IFO-3806, *Salmonella enterica* serovar Typhimurium χ 3306, *Pseudomonas aeruginosa* ATCC 7853, *Serratia marcescens*, *S. sonnei*, and *Proteus mirabilis*. Fungal strains included *Candida albicans* and *Trichophyton* sp.

2.3 Test compounds

We tested the following 14 isoprenoids: geraniol, geranic acid, geranyl acetate, geranyl formate, farnesol, farnesal, citronellol, citral, linalool, linalyl acetate, citronellal, 2,6-diepoxygeraniol, nerol, terpinen-4-ol, as well as hinokitiol and ampicillin as controls (Figure 1). These controls were purchased from Tokyo Chemical Industry Co., Ltd..

All compounds tested were determined to be >90% pure by HPLC analysis.

Figure 1. Isoprenoid and controls

3. RESULTS

3.1. Effects of isoprenoids on Gram-positive bacteria

As shown in Table 1., farnesol exhibited stronger growth-inhibitory effects than geraniol on the two *S. aureus* isolates and *L. monocytogenes*. Citronellol also significantly inhibited these strains' growth. In contrast, linalool and terpinen-4-ol were not active against these Gram-positive bacteria.

Table 1. Effects of isoprenoids on Gram-positive bacteria

	MIC (μ g/ml)		
	<i>S. aureus</i>	MRSA	<i>L. monocytogenes</i>
geraniol	534	534	378
geranic acid	1950	1950	n.a.
geranyl acetate	9200	9200	9200
geranyl formate	9200	9200	7400
2,6-diepoxygeraniol	2000	2000	3900
nerol	394	548	n.t.
farnesol	184	184	133
farnesal	40	40	n.t.
citronellol	279	321	321
citronellal	n.a.	n.a.	n.a.
citral	720	720	720
linalool	1310	1310	n.a.
terpinen-4-ol	1400	1400	n.a.
hinokitiol	n.t.	1.25	0.68
ampicillin	0.4	n.t.	n.t.

n.a., not active; n.t., not tested.

3.2. Effects of isoprenoids on Gram-negative bacteria

As shown in Table 2., geraniol was the only compound that exerted significant growth-inhibitory effects against each of the Gram-negative bacteria tested.

Linalool and terpinen-4-ol were both active against *E. coli* and *S. sonnei*. In contrast, farnesol was not detectably active against Gram-negative bacteria, and citronellol inhibited only *S. sonnei*.

3.3. Effects of isoprenoids on fungi

As shown in Table 3., geraniol strongly inhibited the growth of both *C. albicans* and *Trichophyton* sp. Farnesol again exerted the strongest growth-inhibitory effects on both fungi followed by geraniol, and citral.

Table 2. Effects of isoprenoids on Gram-negative bacteria

	MIC ($\mu\text{g/ml}$)				
	<i>E. coli</i>	<i>S. sonnei</i>	<i>S. Typhimurium</i>	<i>S. marcescens</i>	<i>P. mirabilis</i>
geraniol	445	445	400	422	400
geranic acid	n.a.	n.a.	n.t.	n.a.	n.t.
geranyl acetate	n.a.	9200	n.a.	n.a.	n.a.
geranyl formate	n.a.	n.a.	n.a.	n.a.	7400
2,6-diepoxygeraniol	9800	7800	9800	n.a.	7800
nerol	613	394	n.t.	n.t.	n.t.
farnesol	n.a.	n.a.	n.a.	n.a.	n.a.
farnesal	n.a.	n.t.	n.t.	n.t.	n.t.
citronellol	n.a.	471	n.a.	n.a.	n.a.
citronellal	n.a.	n.a.	n.t.	n.a.	n.t.
citral	1800	900	1800	7200	900
linalool	633	655	1746	1746	1746
terpinen-4-ol	676	700	1866	1866	1866
hinokitiol	34	n.t.	34	n.t.	n.t.
ampicillin	4.0	n.t.	n.t.	n.t.	n.t.

n.a., not active; n.t., not tested.

Table 3. Effects of isoprenoids of *fungi*

	MIC ($\mu\text{g/ml}$)	
	<i>C. albicans</i>	<i>Trichophyton</i> sp.
geraniol	350	88
geranic acid	750	7.5
geranyl acetate	3700	370
geranyl formate	1900	190
2,6-diepoxygeraniol	3900	200
nerol	n.t.	n.t.
farnesol	35	3.5
farnesal	680	34
citronellol	340	34
citronellal	340	170
citral	360	72
linalool	1700	170
terpinen-4-ol	n.t.	n.t.
hinokitiol	n.t.	17
ampicillin	n.t.	n.t.

n.a., not active; n.t., not tested.

Values $\geq 800 \mu\text{g/ml}$ are considered negative.

4. DISCUSSION

When we compared the isoprenoids' effects against Gram-positive and Gram-negative bacteria, farnesol, farnesal, and citronerol inhibited Gram-negative bacterial growth to a greater extent. In contrast, linalool and terpin-4-ol were more effective against Gram-negative bacteria than Gram-positive strains. There are many reports describing the antimicrobial activities of essential oils [26-30].

In contrast to these studies, Pattnaik's group [31] analyzed individual essential oil components, demonstrating that linalool exerted antimicrobial activity against Gram-positive and Gram-negative bacteria. In contrast, we found here that linalool unexpectedly inhibited only Gram-negative bacteria (Tables 1 and 2). We will also explore this possibility using essential oil extracts.

In general, MIC values determined for the isoprenoids were greater than those of the positive controls, ampicillin, and hinokitiol [32, 33]; however, the activities of geranic acid or farnesol were essentially equivalent to that of hinokitiol against *Trichophyton* sp. It will also be of great interest to determine the combined effects of isoprenoids and antibiotics, as has been reported by others [34-36].

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We are also exploring the possibility that these isoprenoids act synergistically, and are encouraged by our initial results.

Finally, we will investigate the mechanisms responsible for the antimicrobial actions of these isoprenoids.

5. CONCLUSIONS

We determined the antimicrobial activities of selected isoprenoids.

Geraniol exhibited a broad spectrum of antibiotic activity, and significantly inhibited the growth of Gram-positive *S. aureus* 209P and an MRSA strain, and the Gram-negatives *E. coli*

IFO-3806 and *S. sonnei*. Farnesol inhibited *S. aureus* strains 209P and 834 to a greater extent than did geraniol, but did not detectably inhibit Gram-negative bacteria

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