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RESEARCH NOTE

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Antibacterial Activity of Panduratin A and Isopanduratin A Isolated from *Kaempferia pandurata* Roxb. against Acne-causing Microorganisms

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Abstract *Propionibacterium acnes* is the predominant organism in sebaceous regions of the skin and is thought to play an important role in the pathogenesis of inflamed lesions. Antibacterial compounds against *P. acnes* were isolated from the ethanol extract of *Kaempferia pandurata* Roxb. and identified as panduratin A and isopanduratin A. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of panduratin A for *P. acnes* were 2 and 4 μg/mL, respectively, while those of isopanduratin A were 4 and 8 μg/mL, respectively. The time-dependent killing effect showed that panduratin A and isopanduratin A completely inhibited the growth of *P. acnes* at 4 and 8 μg/mL in 48 hr, respectively. Panduratin A and isopanduratin A demonstrated high antibacterial activities not only against *P. acnes* but also other skin microorganisms. The results suggest that panduratin A and isopanduratin A could be employed as natural antibacterial agents to inhibit the growth of acne and skin disease causing microorganisms.

Keywords: antibacterial, panduratin A, isopanduratin A, *Kaempferia pandurata* Roxb., *Propionibacterium acnes*, skin disease microorganism

Introduction

Acne, an inflammatory disease of the sebaceous glands, is a common skin disease that induces inflammation at the skin surface of the face, neck, chest, or back. Especially, *Propionibacterium acnes* is one of major organisms isolated from the surface of acne skin (1). Lipase secreted by *P. acnes* degrades sebum oils into free fatty acids, which stimulate the hair follicle, form the comedo, and then induce the inflammation (2). Therefore, *P. acnes* is considered to play an important role in acne development by secreting inflammation-inducing factors.

Compounds targeting acne vulgaris should be able to inhibit *P. acnes* and reduce pro-inflammatory lipids in sebum. Although topical and oral antibacterials such as clindamycin, erythromycin, and tetracycline can reduce the population of *P. acnes* and exert anti-inflammatory actions, these conventional agents also possess adverse effects such as skin irritation, erythema, dryness, and peeling (3,4). To overcome adverse effects of antibiotics, medicinal plants have been extensively studied as alternative treatments for acne diseases.

Kaempferia pandurata Roxb. is a perennial herb of Zingiberaceae (Ginger family) cultivated in tropical countries, including Indonesia and Thailand. The fresh rhizome has been used as a food ingredient and as a folk medicine for the treatment of colic disorder, aphrodisiac, dry cough, rheumatism, and muscular pains. Several studies have reported various activities of *K. pandurata*, including antibacterial, anti-inflammatory, antitumor, antidiarrhea, antidysentery, and antiepidermophytid effects (5-11). However, antibacterial activity of *K. pandurata* against *P. acnes* and skin microorganisms has been rarely investigated. This study aimed to isolate and characterize the antibacterial compounds from *K. pandurata* against acne-causing and skin disease bacteria.

Materials and Methods

Plant material Dried rhizomes of *Kaempferia pandurata* Roxb. were collected in Jakarta, Indonesia, and identified by Dr. Baek NI, Department of Oriental Medicinal Materials and Processing, Kyunghee University (Yongin, Korea). A voucher specimen is deposited in the Department of Biotechnology, Yonsei University (Seoul, Korea).

Isolation of panduratin A and isopanduratin A The ground *K. pandurata* Roxb. (100 g) was extracted with 95% ethanol (400 mL), and the extract (11.95 g) was further fractionated with ethyl acetate. The ethyl acetate fraction was applied to a silica gel column (70-230 mesh, Merck & Co., Whitehouse Station, NJ, USA) and eluted with *n*-hexane, chloroform, and ethyl acetate solution (15:5:1.5, v/v/v) to give 7 fractions (fraction 1 to fraction 7). Fraction 3 (1.51 g) was further separated with *n*-hexane, ethyl acetate, and methanol solution (18:2:1, v/v/v), yielding fraction 3-B (1.1 g). Fraction 3-B was eluted with 100% methanol using preparative high performance liquid chromatography (HPLC, column: W-252, 20.0 mm i.d.× 500 mm L, Japan Analytical Industry Co., Ltd., Tokyo, Japan), and compound 3-B (0.9 g) was finally obtained as

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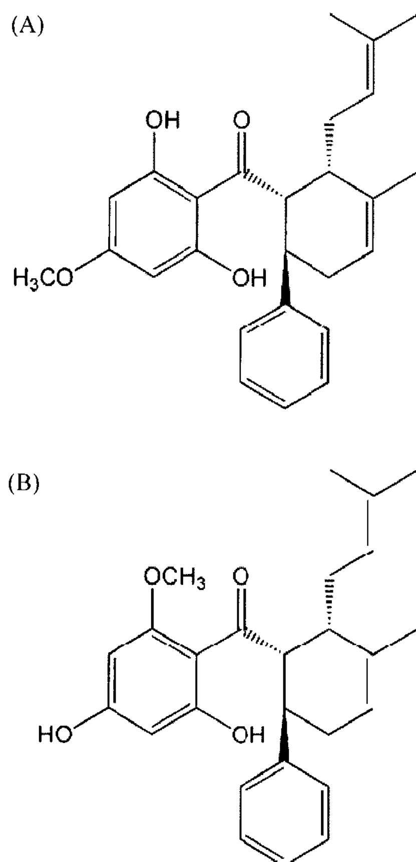

Fig. 1. The chemical structure of (A) panduratin A and (B) isopanduratin A.

a single compound. Careful comparison of several spectral data of compound 3-B including ^{13}C-nuclear magnetic resomance (NMR), ^{1}H-NMR, ^{13}C-DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, and FAB-mass spectra (MS) with those in the literature suggested the chemical structure to be panduratin A (Fig. 1A) or (4-methoxy-2,6-dihydroxyphenyl)-[3'-methyl-2'-(3''-methybut-2''-enyl)-6'-phenylcyclohex-3'-enyl] methanone (5,9). Fraction 4 (1.09 g) was eluted with 90% methanol by Rp-18 column chromatography (LiChropep, 25-40 mm, Merck & Co.), yielding fraction 4-B (0.81 g). Then, fraction 4-B was eluted with chloroform and methanol solution (10:0.2, v/v), yielding fraction 4-B-2 (0.65 g). Fraction 4-B-2 was eluted with *n*-hexane and ethyl acetate solution (10:3, v/v), yielding single compound 4-B-2-2 (0.57 g). Careful comparison of several spectral data of compound 4-B-2-2 including ^{13}C-NMR, ^{1}H-NMR, ^{13}C-DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, and FAB-MS with those in the literature suggested the chemical structure to be isopanduratin A (Fig. 1B) or (2-methoxy-4,6-dihydroxyphenyl)-[3'-methyl-2'-(3''-methybut-2''-enyl)-6'-phenylcyclohex-3'-enyl]methanone (6,9).

Instrumentation NMR spectra were recorded on a Bruker Avance-600 spectrometer (Rheinstein, Germany) at 600 MHz for ^{1}H- and ^{13}C- in $CDCl_3$ with tetramethylsilane (TMS) as an international standard. Complete proton and carbon assignments were based on 1D (^{1}H-, ^{13}C-, ^{13}C-DEPT) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) NMR experiments. FAB-MS were measured using JMS-700 (Jeol Ltd., Tokyo, Japan). All instrumental data are available upon request.

Test microorganisms and cultures *Propionibacterium acnes* ATCC 6919, *Staphylococcus epidermidis* ATCC 12228, and *Micrococcus luteus* ATCC 10240 were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). *Staphylococcus aureus* KCCM 11764 was supplied from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). *Staphylococcus warneri* (KCTC 3340), *Staphylococcus haemolyticus* (KCTC 3341), *Staphylococcus xylosus* (KCTC 3342), *Staphylococcus hominis* (KCTC 3343), *Staphylococcus saprophyticus* (KCTC 3345), *Acinetobacter johnsonii* (KCTC 12405), and *Acinetobacter johnsonii* (KCTC 12407) were purchased from the Korean Collection for Type Culture (KCTC, Daejeon, Korea). *P. acnes* was cultured in brain heart infusion (BHI, Difco, Detroit, MI, USA) broth at 37°C for 48 hr anaerobically. Muller-Hinton (MH, Difco) broth was used for the culture of *A. johnsonii, M. luteus, S. epidermidis, S. aureus, S. warneri, S. haemolyticus, S. xylosus, S. hominis*, and *S. saprophyticus* at 37°C for 24 hr.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) A 96-well round-bottom microtiter plate, each filled with 100 μL of respective broth, was used to measure MIC. Samples (0.001 g) dissolved in 1% dimethyl sulfoxide (DMSO) were added to the first well and serially diluted with 2-fold dilution method (12,13) to give a concentration range of 500 to 1 μg/mL. A bacterial suspension (100 μL) containing 2×10^5 CFU/mL was added to each well and incubated at 37°C for 24 hr. MIC was determined by judging visually the bacterial growth in the series of wells. MBC was the concentration in which *P. acnes* was unable to remain viable. The control included an inoculated growth medium without the test compounds and some commonly available antibacterial agents used for acne control were employed as positive controls. All experiments were performed in duplicate and the average values were reported as MIC and MBC.

Time-kill curves Microorganism used for time-kill curve of antibacterial compound was *P. acnes*. Time-kill curves were performed in BHI medium for *P. acnes*. Before the test was performed, *P. acnes* was subcultured on agar plates at least twice to activate at 37°C for 24 hr according to their optimum culture condition. Suspension of *P. acnes* was washed 3 times by 50 mM potassium phosphate buffer of pH 7.0 solution and diluted to give a final concentration of 1×10^6 CFU/mL. Each concentration of panduratin A and isopanduratin A was diluted in BHI medium and final concentrations of panduratin A and isopanduratin A were 1×MIC, 2×MIC, 4×MIC, and 8×MIC against *P. acnes*. Cultures were aliquot in microtubes (Axygen Scientific, Inc., Union City, CA, USA) and incubated in shaking water bath (Jeio Tech, Gimpo, Korea) with agitation (200 rpm) at 37°C according to their optimum growth condition. At predetermined time points which are 0, 4, 8, 16, 24, and 48 hr for *P. acnes*, each microtube was taken out and 100 μL aliquots were removed and transferred to microtubes. Incubated mixtures were centrifuged (3,900×g) at 4°C for

Table 1. Comparison of MIC and MBC of panduratin A, isopanduratin A, and commercial antibacterial agents against *Propionibacterium acnes*

Compound	MIC[1)]	MBC[2)]
Panduratin A	2	4
Isopanduratin A	4	8
Clindamycin	2	4
Erythromycin	1	2
Triclosan	31	63

[1)]Minimum inhibitory concentration (μg/mL).
[2)]Minimum bactericidal concentration (μg/mL).

1 min and pellets were washed twice with 0.9 mL of 50 mM potassium phosphate buffer of pH 7.0 solution to obtain panduratin A and isopanduratin A free cells. Pellets were suspended in 100 μL of 50 mM potassium phosphate buffer solution and serially diluted. Twenty-five μL was spread on BHI agar plates for *P. acnes* and incubated at 37°C for 48 hr to determine the number of viable cells (CFU/mL).

Results and Discussion

Antibacterial activity against *P. acnes* It is well known that *P. acnes* plays a central role in acne vulgaris, an inflammatory skin disease (14). Antibacterial activities of panduratin A and isopanduratin A against *P. acnes* were investigated in terms of MIC and MBC in comparison with some commercial agents. MIC and MBC of panduratin A against *P. acnes* were 2 and 4 μg/mL, respectively, while those of isopanduratin A were 4 and 8 μg/mL, respectively (Table 1). MIC and MBC of 2 compounds were much lower than those of a commercial antibacterial agent, triclosan (31 and 63 μg/mL, respectively), indicating that panduratin A and isopanduratin A confer much stronger antibacterial activity than triclosan. Triclosan has been used for the last few decades as a broad-spectrum hydrophobic antibacterial agent in a number of products as diverse as detergents and household products (15). However, it was reported that triclosan also resulted in reducing immune defense and disrupting normal ecology of skin (16). MIC and MBC of 2 compounds were almost comparable to those of antibiotics such as clindamycin (2 and 4 μg/mL, respectively) and erythromycin (1 and 2 μg/mL, respectively). There are several side effects of antibiotics such as appearance of resistant bacteria, organ damage, and immunohypersensitivity if they have been taken for a long time (17). Thus, panduratin A and isopanduratin A can be potent natural agents replacing synthetic antibacterial chemicals and antibiotics. Figure 2 shows the time-dependent killing effect of panduratin A (Fig. 2A) and isopanduratin A (Fig. 2B) on *P. acnes* at various concentrations. It is noteworthy that 4 μg/mL of panduratin A and 8 μg/mL of isopanduratin A completely inhibited the growth of *P. acnes* in 4 hr. These fast bactericidal activities of panduratin A and isopanduratin A in time-kill curve against *P. acnes* are of practical significance, since antibiotics are very limited for extended applications in acne care due to adverse effects such as skin irritation, erythema, dryness, peeling, and environmental pollution (3,4).

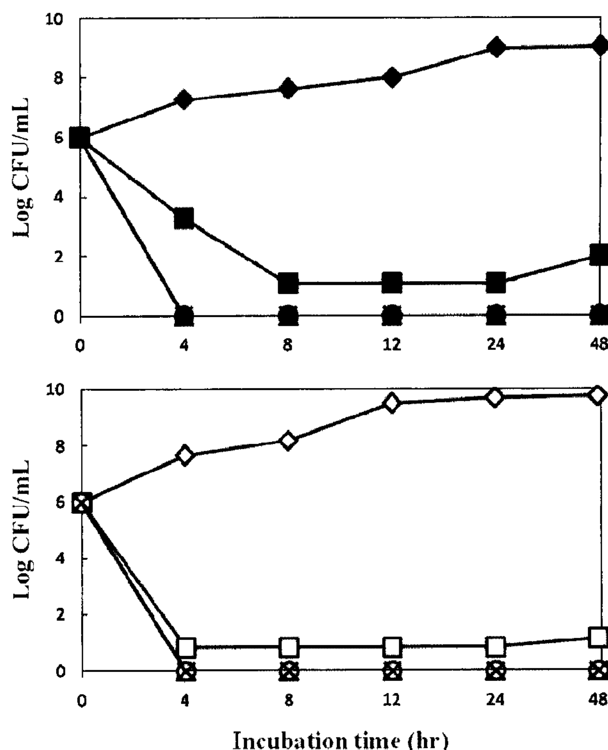

Fig. 2. *In vitro* time-dependent killing effect of (A) panduratin A and (B) isopanduratin A against *P. acnes*. (A): ◆, Control; ■, 2 μg/mL; ▲, 4 μg/mL; ●, 8 μg/mL; X, 16 μg/mL. (B): ◇, Control; □, 4 μg/mL; △, 8 μg/mL; ○, 16 μg /mL; X, 32 μg/mL.

Antibacterial activity against skin microorganisms Panduratin A and isopanduratin A also showed strong antibacterial spectrum against other skin microorganisms such as *S. epidermidis*, *S. aureus*, *S. warneri*, *S. haemolyticus*, *S. xylosus*, *S. hominis*, *S. saprophyticus*, and *M. luteus*, with MIC and MBC ranges of 2-8 μg/mL (Table 2). In contrast, no susceptible activity was noted for *A. johnsonii* and *A. lwoffii* (MIC>500 μg/mL). The results suggest that both panduratin A and isopanduratin A with broad antibacterial spectrum can be employed as efficient topical therapeutic agents for acne and other skin diseases.

In summury, panduratin A and isopanduratin A isolated from the rhizomes of *K. pandurata* Roxb. showed potent antibacterial activities in terms of high efficacy, broad antibacterial spectrum and fast effectiveness for acne causing and skin microorganisms. However, their exact antibacterial mechanisms remain to be clarified and the use of standard skin microorganisms may not truly reflect the susceptibility of clinically isolated microorganisms. Thus, further research is necessary to determine anti-acne activity of panduratin A and isopanduratin A against clinical isolates *in vivo* research model in parallel with mechanistic studies.

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Table 2. MIC and MBC of panduratin A and isopanduratin A against skin microorganisms

	Skin microorganism	Panduratin A		Isopanduratin A	
		MIC[1)]	MBC[2)]	MIC	MBC
Gram (+)	*Micrococcus luteus*	2	4	4	8
	Staphylococcus aureus	2	4	4	4
	Staphylococcus epidermidis	2	4	4	8
	Staphylococcus haemolyticus	2	4	8	8
	Staphylococcus hominis	2	2	2	4
	Staphylococcus saprophyticus	2	4	2	4
	Staphylococcus warneri	2	4	2	4
	Staphylococcus xylosus	4	8	4	4
Gram (-)	*Acinetobacter johnsonii*	>500	>500	>500	>500
	Acinetobacter lwoffii	>500	>500	>500	>500

[1)]Minimum inhibitory concentration (μg/mL).
[2)]Minimum bactericidal concentration (μg/mL).

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