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In vitro antibacterial and anti-inflammatory effects of honokiol and magnolol against *Propionibacterium* sp.

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Abstract

Honokiol and magnolol, two major phenolic constituents of *Magnolia* sp., have been known to exhibit antibacterial activities. However, until now, their antibacterial activity against *Propionibacterium* sp. has not been reported. To this end, the antibacterial activities of honokiol and magnolol were detected using the disk diffusion method and a two-fold serial dilution assay. Honokiol and magnolol showed strong antibacterial activities against both *Propionibacterium acnes* and *Propionibacterium granulosum*, which are acne-causing bacteria. The minimum inhibitory concentrations (MIC) of honokiol and magnolol was 3–4 μg/ml (11.3–15 μM) and 9 μg/ml (33.8 μM), respectively. In addition, the killing curve analysis showed that magnolol and honokiol killed *P. acnes* rapidly, with 10^5 organisms/ml eliminated within 10 min of treatment with either 45 μg (169.2 μM) of magnolol or 20 μg (75.2 μM) of honokiol per ml. The cytotoxic effect of honokiol and magnolol was determined by a colorimetric (3-(4,5-dimetyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) assay using two animal cell lines, human normal fibroblasts and HaCaT. In this experiment, magnolol exhibited lower cytotoxic effects than honokiol at the same concentration, but they showed similar cytotoxicity when triclosan was employed as an acne-mitigating agent. In addition, they reduced secretion of interleukin-8 and tumor necrosis factor α (TNF-α) induced by *P. acnes* in THP-1 cells indicating the anti-inflammatory effects of them. When applied topically, neither phenolic compound induced any adverse reactions in a human skin primary irritation test. Therefore, based on these results, we suggest the possibility that magnolol and honokiol may be considered as attractive acne-mitigating candidates for topical application.

Keywords: Antibacterial; Anti-inflammatory; MIC (Minimum inhibitory concentration); MBC (Minimum bactericidal concentration); Irritation

1. 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. Acne develops mostly in young people due to several factors, namely, hormonal imbalance, bacterial infection, stress, food, or cosmetic application. Especially, *Propionibacterium acnes* which is one of major organisms isolated from the surface of skin (Marples, 1974), induces an inflammation in the sebaceous glands or hair pore (Webster et al., 1978). *P. acnes* secretes lipase and degrades sebum oils into free fatty acids, which are potent acne stimuli. These free fatty acids stimulate the hair follicle, form the comedo, and then induce the inflammation (Downing et al., 1986). These bacteria also secrete leukocyte chemotatic factors, infiltrating leukocytes in the hair follicle. These leukocytes stimulate and destroy the hair follicle wall. Subsequently, the contents of the hair follicle flows into the dermis (Webster et al., 1980). Therefore, *P. acnes* is considered to play an important role in acne development by secreting inflammation-inducing factors.

As therapeutic agents for acne, antibiotics are usually employed to inhibit inflammation or kill the bacteria (Guin et al., 1979). For example, triclosan, benzoyl peroxide, azelaic acid (Breathnach et al., 1984), retinoid, tetracycline, erythromycin, macrolide, and clindamycin are among these

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antibiotics. However, these antibiotics have been known to induce side effects. Benzoyl peroxide and retinoid bring about xerosis cutis and skin irritation if they are used excessively as treatments (Zesch, 1988) and several reports also suggest that in case of tetracycline, erythromycin, macrolide and clindamycin, there are several side effects such as appearance of resistant bacteria, organ damage, and immunohypersensitivity if they have been taken for a long time (Eady, 1998; Wawruch et al., 2002). In addition, triclosan is converted into an environmental hormone when exposed to light, inducing severe environmental pollution (CTFA Safety Testing Guideline, 1991). Therefore, many researchers have tried to develop therapeutic agents for acne, that have no side effects, but high antibacterial activity (Marzulli and Maibach, 1991; Nam et al., 2003; Park et al., 2001; Tan, 2003).

Magnolol and honokiol are isolated from the stem bark of *Magnolia* sp. which has been used as treatments for cough, diarrhea, and allergic rhinitis, etc. as an Oriental medicine in Korea, China, and Japan. Several recent reports identified and proved that magnolol and honokiol have several medicinal functions such as an anxiolytic, anti-inflammatory effect (Guin et al., 1979; Marzulli and Maibach, 1991; Shin et al., 2001; Wang et al., 1995) and inhibitory effect on skin tumor promotion (Shin et al., 2001; Ikeda and Nagase, 2002). In addition, magnolol and honokiol have been known to show high antimicrobial activity against several microorganisms (Bang et al., 2000; Chang et al., 1998; Clark et al., 1981; Ho et al., 2001).

Based on the previously known anti-inflammatory and antibacterial effects of magnolol and honokiol, we investigated the possibility that they may be effective acne treatments. In this paper, we demonstrated that magnolol and honokiol have high antibacterial and anti-inflammatory effects against acne-inducing bacteria and induce no irritating effects in human skin model. Therefore, according to these results, we suggest that magnolol and honokiol may be employed as effective therapeutic agents to ameliorate acne disease.

2. Materials and methods

2.1. Reagents

Heat-killed *P. acnes* was prepared by heating at 95 °C for 5 min and phenolic compounds, honokiol (98.0%) [3′,5-di-2-propenyl-(1,1′-biphenyl)-2,4′-diol] and magnolol (99.0%) [5′,5-di-2-propenyl-(1,1′-biphenyl)-2,2′-diol], were purchased from Wako (Japan). Magnolol and honokiol were dissolved in 10% dimethylsulfoxide (DMSO).

2.2. Cells and cell culture

The human monocytic THP-1 cell line was obtained from ATCC (TIB 202). The THP-1 cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640) (Hyclone) containing 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). The THP-1 cells were incubated with *P. acnes* in 24-well plates at 1×10^6 cells per ml for 24–72 h. Human keratinocytes HaCaT (epithelial cell line from adult human skin) and human normal fibroblast cells (derived from neonatal foreskin) were obtained from Amore-Pacific R&D Center in Korea and cultured in Dulbecco's modified Eagles medium (Hyclone) containing 10% fetal bovine serum (GibCo), and penicillin–streptomycin at 37 °C in a humidified 95% air/5% CO_2 atmosphere.

2.3. Bacteria

We used two kinds of bacteria that cause acne, *P. acnes* ATCC 6919 and *Propionibacterium granulosum* ATCC 25564. Both strains were cultured at 37 °C for 24 h with GAM semisolid medium (GAM) (Nissui, Japan) under anaerobic conditions before the assay.

2.4. Antibacterial test

Antibacterial activity was evaluated using the standardized filter-paper disc-agar diffusion method, known as the Kirby-Bauer method. *P. acnes* and *P. granulosum* were employed in this experiment. Bacterial cells were cultured at 37 °C with brain heart infusion broth under anaerobic conditions until their growth stage reached a stationary phase. Approximately 10^6 bacterial cells were inoculated into 7 ml GAM agar medium (containing 0.8% phytoagar), and then GAM agar medium containing 10^6 bacterial cells was poured onto the GAM agar plate (containing 1.5% phytoagar). After that, filter-paper discs of uniform size (diameter of 10 mm) are impregnated with specified concentrations of test materials and then placed on the surface of an agar plate that has been seeded with the organism to be tested. The plates were then incubated at 37 °C for 24 h under anaerobic conditions. Antibacterial activity was defined by measuring the diameter of the growth inhibition zone. Erythromycin was employed as the positive control.

2.5. Determination of the minimum inhibitory concentration (MIC)

We pre-cultured the bacterial cells for 24 h at 37 °C in 10 ml brain heart infusion broth under anaerobic conditions. About 10^4 bacterial cells of the pre-cultured bacteria were inoculated into 3 ml of brain heart infusion broth. Subsequently, honokiol and magnolol were then added into the 3 ml brain heart infusion broth containing the bacteria and cultured for 24 h at 37 °C under anaerobic conditions. To determine the MIC of honokiol and magnolol, we employed a two-fold serial dilution method. The MIC value was defined as the lowest concentration that yield no bacterial cell growth.

2.6. Determination of the minimum bactericidal concentration (MBC)

MBC was determined according to NCCLS guidelines (National Committee for Clinical Laboratory Standards, 1987). The killing curve assay was performed on the basis of a previously published standard protocol (Lorian, 1996), and the experiment was performed in duplicate. *P. acnes* from the logarithmic phase of growth were collected and they were incubated at 37 °C with different concentrations of magnolol or honokiol in a 5 ml total volume of cation-adjusted MHB (10^5 to 10^6 organisms/ml) for 10 min, 30 min, 1 h, or 2 h, respectively. After incubation with magnolol or honokiol for the indicated time, 0.1 ml of the culture (5 ml) was collected and was mixed with 25 ml molten agar for the preparation of agar pour plates. Since reagents were diluted at least 250-fold in the plates, the reagent carryover effect was minimal. In addition, to obtain the appropriate numbers of Colony Forming Unit (CFU) in an individual plate (fewer than 150 colonies/plate) to ensure accurate colony counting, 0.2 ml of the culture (5 ml) was taken at different time points, and a series of 10-fold dilutions (10^{-1} to 10^{-7}) was prepared. Then 0.1 ml of the diluted cells was used to prepare the plates as described above. The plates were incubated overnight at 37 °C.

2.7. Measurement of cytokine production

Human monocytic THP-1 cells (1×10^6) in serum-free medium were stimulated with *P. acnes* 100 μg/ml alone or combination of indicated concentrations of magnolol or honokiol, and were incubated for 48 h. The culture supernatants were then harvested after 48 h for interleukin-8 or tumor necrosis factor alpha (TNF-α). Concentrations of interleukin-8 and TNF-α in the culture supernatant were measured using Enzyme-linked Immunosorbent Assay (ELISA) kits (Genzyme, Minneapolis, MN). Culture supernatants were added to 96 wells and then diluted biotinylated anti-interleukin-8 or TNF α was added to the sample wells. After incubation for 3 h at room temperature, the sample wells were washed. Streptavidin-HRP was distributed to the sample wells and the plate was then incubated 30 min at room temperature. After incubation, all wells were washed and 3, 3′, 5, 5′-tetramethylbenzidine (TMBZ) substrate solution was added. After incubation in the dark, absorbance was read at 450 nm for 12–15 min according to the manufacture's instructions. Data are expressed as means ± S.E.M.

2.8. Cytotoxicity assay

HaCaT and human normal fibroblast cells were cultured in Dulbecco's modified Eagles medium (Hyclone) containing 10% fetal bovine serum (GibCo), and penicillin–streptomycin at 37 °C in a humidified 95% air/5% CO_2 atmosphere. Cells were seeded on 24-well plates and drug treatment began 24 h after seeding. General viability of cultured cells was determined by the reduction of 3-(4,5-dimetyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to formazan (Mosmann, 1983). The MTT assay was performed after the incubation of HaCaT and normal fibroblast cells treated with various concentrations of magnolol, honokiol, and triclosan, respectively, for 24 h at 37 °C in 5% CO_2 atmosphere, MTT (1 mg/ml in Phosphate-buffered saline, PBS) was added to each well in a 1/10 volume of media. Cells were incubated at 37 °C for 3 h, and dimethylsulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance was then measured at 570 nm with a spectrophotometer (Power Wave, Bio-tek).

2.9. Human skin primary irritation test

Thirty healthy Korean subjects were studied, and written consent was obtained in each case. The average age was 22.1 years (range 20 to 27: all females). The subjects had no skin disease, nor had they used topical or systemic irritant preparations in the previous 1 month. All volunteers were nonsmokers. The subjects received test materials which were formulated with petrolatum. The primary skin irritation test was performed using a Finn Chamber® secured to the back site with Scanpore tape. These chambers are made of inflexible aluminum, and have a diameter of 8 mm and a depth of 0.5 mm. The round border of the chamber was placed firmly against the skin, causing a tight occlusion of the test materials. Magnolol plus honokiol (ratio of 1:1) or honokiol formulated with petrolatum was prepared and applied. The patches (chambers) stayed in place for 48 h. The subjects abstained from showering or performing any work or exercise that might wet or loosen the patches. Once the patches were removed, a reading was done after 30 min and 1 day later. A reading was scored according to the criteria of the International Contact Dermatitis Research Group (ICDRG).

2.10. Statistical evaluation

Averages ± S.E.M. of the means were calculated; statistical analysis of results was performed by Student's *t*-test for independent samples. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Antibacterial test of magnolol and honokiol

To elucidate the antibacterial activity of honokiol and magnolol against *P. acnes* and *P. granulosum*, we introduced a disk diffusion method (Table 1). Erythromycin was employed as a positive control. Honokiol and magnolol

Table 1
Antibacterial activity of honokiol and magnolol against *Propionibacterium* sp. by disk diffusion method

Compounds	Diameter of clear zone (mm)			
	6.25	12.5	25	50[a]
P. acnes				
Honokiol	11 ± 0.1[b]	13 ± 0.7[b]	16 ± 0.4[b]	20 ± 1.4[b]
Magnolol	–[c]	11 ± 0.2[b]	12 ± 0.1[b]	15 ± 0.5[b]
Erythromycin	40 ± 2.4[b]	40 ± 3.5[b]	42 ± 2.8[b]	46 ± 6[b]
P. granulosum				
Honokiol	13 ± 0.3[b]	15 ± 0.5[b]	18 ± 0.8[b]	24 ± 2.1[b]
Magnolol	–[c]	12 ± 0.2[b]	15 ± 0.9[b]	20 ± 1.3[b]
Erythromycin	42 ± 4.1[b]	44 ± 1.5[b]	46 ± 4.5[b]	50 ± 8.5[b]

[a] Amount of compounds (μg/disk).
[b] $P < 0.05$, diameter of filter disk is 10 mm.
[c] No antibacterial activity.

were found to have significant antibacterial activities against both bacterial cells, although their activities were less potent than those of erythromycin. As shown in Table 1, the inhibitory zones that indicate the antibacterial activities of honokiol and magnolol, respectively against both *P. acnes* and *P. granulosum* were conspicuously detected at over 6.25 μg/disk (0.39 mM) of honokiol and 12.5 μg/disk (0.78 mM) of magnolol. The diameter of these inhibitory zones were a linear function of the logarithmic concentration within a range of 6.25–200 μg/disk (0.39–12.48 mM; Fig. 1).

3.2. Determination of MIC of magnolol and honokiol against Propionibacterium sp.

The antibacterial activities of both magnolol and honokiol were further evaluated by determining the minimum inhibitory concentration (MIC), which is the lowest concentration yielding no growth. The MIC of magnolol and honokiol was determined using a two-fold serial dilution method. As shown in Table 2, while honokiol inhibited the growth of *P. acnes* at 4 μg/ml (15 μM) and *P. granulosum* at 3 μg/ml (11.3 μM), magnolol inhibited the growth of both bacterial cells at 9 μg/ml (33.8 μM). Therefore, the MIC of honokiol and magnolol is 3–4 μg/ml (11.3–15 μM) and 9 μg/ml (33.8 μM), respectively.

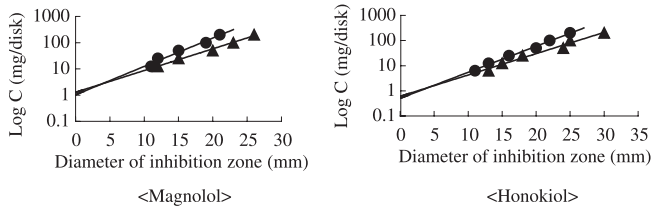
Table 2
Minimum inhibitory concentration (MIC) of honokiol and magnolol against *Propionibacterium* sp.

Microorganism	MIC of agents (μg/ml)	
	Honokiol	Magnolol
P. acnes	4	9
P. granulosum	3	9

3.3. Bactericidal activity of magnolol and honokiol

The difference between MICs and MBCs has been established as an index of the bactericidal activity of antibiotics (Kuribara et al., 2000). *P. acnes* was tested to determine the MBCs of magnolol and honokiol. The kinetics of bacterial killing were evaluated at three concentrations (1×, 5×, 10× of the MIC) of magnolol or honokiol. The bacterial cell viability within the first 2 h was measured. As shown in Fig. 2, the rate of bacterial killing was dependent on the concentration of magnolol or honokiol, and showed more than 10^5 organisms/ml being eradicated (reduction to <4 CFU/ml) within 2 h at the highest concentration studied. Bacterial cultures were monitored for up to 24 h, and no regrowth was observed; for the species (*P. acnes*) tested, no colony was found after 0.2 ml of the 24 h culture was plated. The best activity rate was identified when more than 10^6 *P. acnes* organisms per ml were eradicated within 10 min with either 20 μg (75.2 μM) of honokiol per ml or 45 μg (169.2 μM) of magnolol.

A



B

	Magnolol		Honokiol	
P. acnes (●)	$y = 1.0301e^{0.2489x}$	$R^2=0.9694$	$y=0.5036e^{0.2384x}$	$R^2=0.9925$
P. granulosum (▲)	$y = 1.2829e^{0.1908x}$	$R^2=0.9908$	$y=0.6058e^{0.1952x}$	$R^2=0.9736$

Fig. 1. Standard curve of the inhibitory effects of magnolol and honokiol against *Propionibacterium* sp. (A) and their functional formulas (B). Inhibitory effect of magnolol was measured at 12.5 to 200 μg/disk (0.78 mM to 12.48 mM/disk) and honokiol was measured at 6.25 to 200 μg/disk (0.39 to 12.48 mM/disk).

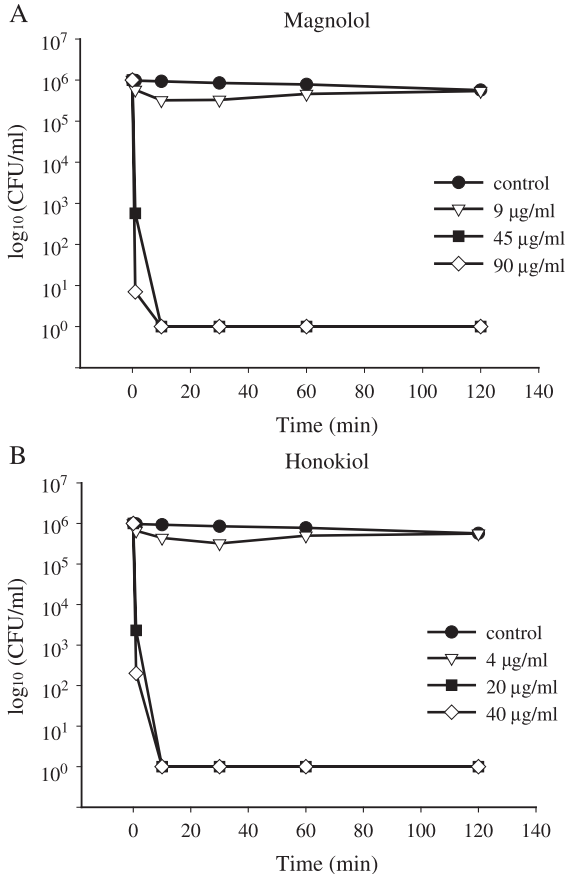


Fig. 2. Killing time curve. The killing activity of magnolol and honokiol against *P. acnes* was monitored for the first 2 h. (A) Magnolol; (B) honokiol. For 20 μg/ml (75 μM) and 40 μg/ml (150 μM) of honokiol or for 45 μg/ml (170 μM) and 90 μg/ml (340 μM) of magnolol, the killing curves were identical (almost overlapping in the figure). Under the conditions used in this study, the lowest detectable level was 4 CFU/ml.

3.4. Magnolol and honokiol inhibited P. acnes-induced secretion of proinflammatory cytokines such as interleukin-8 and TNF-α

It is widely accepted that the main proinflammatory mediators induced by bacteria and their cell components are cytokines, primarily TNF-α, and interleukin-8. Therefore, to investigate anti-inflammatory effects of magnolol and honokiol, we performed ELISA for interleukin-8 and TNF-α in THP-1 cells. As shown in Fig. 3A and B, *P. acnes*-induced production of TNF-α, and interleukin-8 in THP-1 cells was all reduced by magnolol and honokiol. While honokiol inhibited production of TNF-α slightly stronger than magnolol at all test concentrations, in case of interleukin-8, magnolol at 10 μM caused a greater inhibition than honokiol at the same concentration; but in case of 20 μM, magnolol did not inhibited interleukin-8 more potently than honokiol. This result led us to guess that 10 μM of magnolol is optimal concentration in case of interleukin-8.

However, there is the possibility that the reduction of proinflammatory cytokines was induced by cytotoxic effect of magnolol or honokiol. To confirm this, we performed MTT assay in THP-1 cells. According to this result, magnolol and honokiol showed no cytotoxic effect at 20 μM of magnolol or honokiol (data not shown).

3.5. Cytotoxicity assay of magnolol and honokiol in mammalian cell lines

We examined the cytotoxic effects of honokiol and magnolol on both human normal fibroblasts and HaCaT (Fig. 4). In human normal fibroblast cells, magnolol and triclosan at lower concentrations than 5 μg/ml (magnolol: 18.8 μM, triclosan: 17.3 μM) showed no cytotoxicity, whereas honokiol showed 58% cytotoxicity at 5 μg/ml (18.8 μM) in human normal fibroblast cells. Upon treatment of magnolol and triclosan at 10 μg/ml (magnolol: 37.6 μM, triclosan: 34.5 μM), over 45% of human normal fibroblast cells survived; but much lower cell viability (13%) was observed in case of honokiol at 10 μg/ml (37.6 μM). In HaCaT, magnolol at concentrations lower than 10 μg/ml (37.6 μM) showed less than 30% cytotoxicity, whereas honokiol and triclosan at 5 μg/ml (honokiol: 18.8 μM, triclosan: 17.3 μM) showed 52% and 41% cytotoxicity, respectively. However, honokiol and triclosan at 10 μg/ml (honokiol: 37.6 μM, triclosan: 34.5) exhibited fatal cytotoxicity.

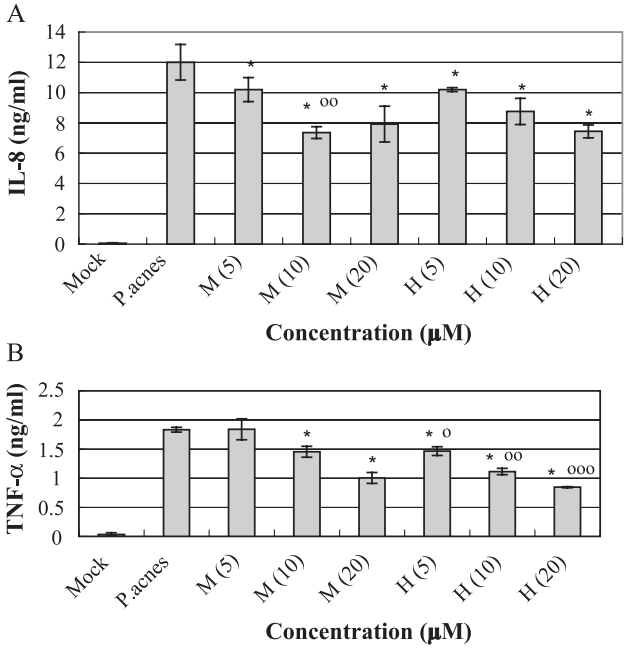


Fig. 3. Magnolol and honokiol inhibited *P. acnes*-induced secretion of proinflammatory cytokines such as interleukin-8 (A) and TNF-α (B). Dose-dependent effect of magnolol and honokiol treatment on *P. acnes*-induced interleukin-8 (A) or TNF-α (B) release. THP-1 cells were stimulated with or without *P. acnes*, and the supernatants were harvested after 48 h for interleukin-8 or TNF-α measurement. Data are expressed as means ± S.E.M *$P<0.05$ compared with *P. acnes*. $^{\circ}P<0.05$ versus M (5) or H (5). $^{\circ\circ}P<0.05$ versus M (10) or H (10). $^{\circ\circ\circ}P<0.05$ versus M (20) or H (20). M: magnolol, H: honokiol.

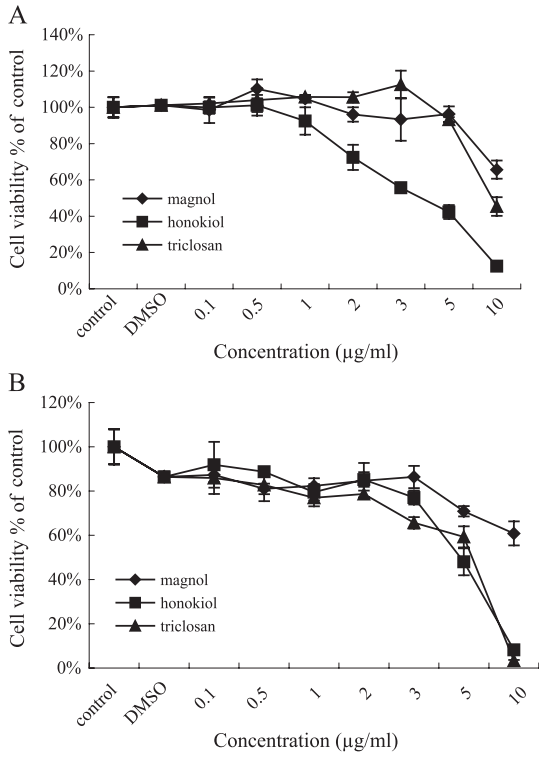


Fig. 4. Cytotoxicity of magnolol and honokiol against human normal fibroblasts (A) and HaCaT (B). Human normal fibroblast cells or HaCaT cells were cultured for 24 h in medium with either presence or absence of test agents. The cellular cytotoxicity was determined according to a rapid colorimetric MTT assay, and expressed as the mean ± S.E.M.

3.6. Human skin primary irritation test of magnolol and honokiol

To evaluate the irritation effect of magnolol and honokiol for clinical applications to human skin, a patch test was performed. In our study, as shown in Table 3, none of the 30 subjects experienced a reaction based on the 30 min and 1 day readings. Specifically, we did not observe any adverse reactions such as erythema, burning, or pruritus in the study subjects that was related to the topical treatment of magnolol and honokiol.

4. Discussion

As previously mentioned, we identified the antibacterial and anti-inflammatory effect of magnolol and honokiol against acne-inducing bacteria; (1) the MIC of honokiol is 3–4 μg/ml (11.3–15 μM) and magnolol is 9 μg/ml (33.8 μM), suggesting that honokiol has more potent activity than magnolol. (2) Bactericidal activity of magnolol and honokiol showed dose dependence. Especially, the best bactericidal activity was identified upon treatment of either 20 μg of honokiol per ml (75.2 μM) or 45 μg of magnolol per ml (169.2 μM). This result indicate that at the appropriate concentrations, both magnolol and honokiol are rapidly acting bactericidal agents. (3) *P. acnes*-induced production of TNF-α, and interleukin-8 in THP-1 cells was all reduced by magnolol and honokiol, indicating that magnolol and honokiol have anti-inflammatory effect. Unlike in human normal fibroblasts (magnolol [5 mg/ml = 18.8 μM]: no cytotoxicity, honokiol [18.8 μM]: 58% cytotoxicity) and HaCaT cells (magnolol [5 mg/ml = 18.8 μM]: 29% cytotoxicity, honokiol [18.8 μM]: 52% cytotoxicity), magnolol and honokiol at 20 μM showed no cytotoxic effect suggesting that cytotoxic effect of magnolol and honokiol does not contribute to inhibition of interleukin-8 and TNF-α production, induced by *P. acnes*. This cytotoxic difference among these animal cell lines is probably attributed to the difference in cell type such as growth pattern and origin of cell line. That is, THP-1 cell line shows anchorage-independent growth pattern and its origin is human monocyte.

However, despite their promising antibacterial and anti-inflammatory effect against acne-inducing bacteria, there was the possibility that when applied as a therapeutic agent for acne, they might have cytotoxic effects on human skin cells. In this case, they could not be clinically introduced as a therapeutic agent for acne. To examine the cytotoxic effects of magnolol and honokiol, an MTT assay was performed in both human normal fibroblasts and HaCaT cells. We found that in addition to having less active antibacterial activity than honokiol, magnolol has weaker cytotoxic effects than honokiol (Fig. 4). As well, like triclosan (2, 4, 4-trichloro-2-hydroxy-diphenylether) which has been used as a good active ingredient for many antibacterial skin-care products, owing to its antibacterial and anti-inflammatory properties (Kuribara et al., 2000; Rogos et al., 1974), both honokiol and magnolol have relatively low cytotoxic effects suggesting the possibility of introducing them as safe topical therapeutic agents for acne. To further confirm the safety of magnolol and honokiol, a patch test was performed. This test confirmed the possibility that magnolol and honokiol may be safely employed in human skin. However, we cannot completely

Table 3
The results of human skin primary irritation test ($n = 30$)

No	Test material	48 h					72 h					Reaction grade		
		±	1+	2+	3+	4+	±	1+	2+	3+	4+	48 h	72 h	Mean
1	Petrolatum	–[a]	–	–	–	–	–	–	–	–	–	0	0	0
2	M[b] + H[c] (10 μg/ml)	–	–	–	–	–	–	–	–	–	–	0	0	0
3	M + H (100 μg/ml)	–	–	–	–	–	–	–	–	–	–	0	0	0
4	H (5 μg/ml)	–	–	–	–	–	–	–	–	–	–	0	0	0
5	H (20 μg/ml)	–	–	–	–	–	–	–	–	–	–	0	0	0
6	H (50 μg/ml)	–	–	–	–	–	–	–	–	–	–	0	0	0

Reaction grade = Σ[{Grade × No. of Responders}/{4(Maximum grade) × 30(Total Subjects)}] × 100 × (1/2).

[a] No reaction.

[b] M: magnolol.

[c] H: honokiol.

exclude the possibility that owing to the short-term treatment of them, they showed no irritation in patch test. Therefore, it is required to demonstrate their properties in case of being applied for many days in human skin.

Based on these results, we can conclude that magnolol and honokiol may be introduced as a possible therapeutic agent for acne. However, although the antimicrobial and anti-inflammatory effect of magnolol and honokiol against acne-inducing bacteria was identified, their action mechanisms was not determined. Especially, their possible inhibition mechanisms of proinflammatory cytokines remain to be evaluated in further study. Until now, NF-κB (nuclear factor-κB) has been reported to be involved in maximal transcription of many cytokines, including TNF-α, interleukin-1, interleukin-6, and interleukin-8, which are thought to be important in the generation of acute inflammatory responses. So, we consider the possibility that magnolol and honokiol inhibit NF-κB activation induced by *P. acnes*.

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