Antibacterial and anti-inflammatory effects of Jeju medicinal plants against acne-inducing bacteria

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Propionibacterium acnes and Staphylococcus epidermidis are pus-forming bacteria that trigger inflammation in acne. The present study was conducted to evaluate the antimicrobial activities of Jeju medicinal plants against these etiologic agents of acne vulgaris. Ethanol extracts of Jeju plants were tested for antimicrobial activities by disc diffusion and broth dilution methods. The results from the disc diffusion assays revealed that four medicinal plants, Mollugo pentaphylla, Angelica anomala, Matteuccia orientalis, and Orika japonica inhibited the growth of both pathogens. Among these, A. anomala had strong inhibitory effects. Its MIC values were 15.6 µg/ml and 125 µg/ml against P. acnes and S. epidermidis, respectively. The cytotoxic effects of the four extracts were determined by colorimetric MTT assays using two animal cell lines: human dermal fibroblasts and HaCaT cells. Although the M. orientalis root extract had moderate cytotoxicity in HaCaT cells at 200 µg/ml, most extracts exhibited low cytotoxicity at 200 µg/ml in both cell lines. In addition, the extracts reduced the P. acnes-induced secretion of interleukin-8 and tumor necrosis factor-alpha (TNF-α) in THP-1 cells, an indication of their anti-inflammatory effects. Based on these results, we suggest that M. pentaphylla, A. anomala, M. orientalis, and O. japonica are attractive acne-mitigating candidates for topical application.

Key Words——acne; cosmetics; interleukin-8; Propionibacterium acnes; Staphylococcus epidermidis; tumor necrosis factor-α

Introduction

Acne vulgaris is the most common skin disease that affects areas containing the largest oil glands, including the face, back, and trunk (Park et al., 2004). Normal skin commensals including Propionibacterium acnes, Propionibacterium granulosum, Staphylococcus epidermidis and Malassezia furfur, proliferate rapidly during puberty and are often involved in the development of acne. Propionibacterium acnes has been described as an obligate anaerobic organism. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. On the other hand, Staphylococcus epidermidis, an aerobic organism, usually involves superficial infections within the sebaceous unit (Chomnawang et al., 2005).

Propionibacterium acnes has been implicated over other cutaneous microflora in contributing to the inflammatory response of acne. It acts as an immuno-stimulator which can produce a variety of enzymes and biologically active molecules, which are involved in the development of inflammatory acne. These products include lipases, proteases, hyaluronidases, and chemotactic factors. The main components of the

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pilosebaceous unit on the skin, such as keratinocytes and sebocytes, can be activated by P. acnes, leading to the production of pro-inflammatory cytokines (Leeming et al., 1985; Vowels et al., 1995). It has been reported that a secreted peptidoglycan of P. acnes can stimulate the production of the proinflammatory cytokines such as interleukin-1 (IL-1), IL-8, and tumor necrosis factor-alpha (TNF-α) by human monocyte cell lines and freshly isolated peripheral blood mononuclear cells from acne patients (Chen et al., 2002; Jain and Basal, 2004).

As therapeutic agents for acne, antibiotics are typically employed to inhibit inflammation or kill bacteria. Triclosan, benzoyl peroxide, azelaic acid, salicyl, tetracycline, erythromycin, roxithromycin, and clindamycin are among these antibiotics. However, antibiotic resistance has been increasing in prevalence within the dermatologic setting (Swanson, 2003). The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. Traditional herbal medicines provide an interesting, largely unexplored source for new drug development. Indeed, the potential use of traditional herbal medicines as a basis for new skin-care cosmetics has been emphasized recently (Kim et al., 2007). It is of great interest to determine whether preparations used cosmetically in folk medicine have activities that could be useful in modern formulations. In our efforts to find new functional ingredients for anti-acne preparations, 100 medicinal plant extracts were examined for antimicrobial and anti-inflammatory activity against P. acnes and S. epidermidis.

Materials and Methods

Uses in traditional medicine and previously isolated constituents. Traditional usage and isolated substances from the selected medicinal plants are listed in Table 1.

Plant material and extraction. An ethnobotanical survey was carried out on Jeju Island of South Korea from July to October 2005. Freshly picked plants were air-dried at room temperature for 2 weeks with no direct sunlight. Voucher specimens were identified by Dr. G. Kim and deposited in the Jeju Bio-Industry Development Center of the Jeju Hi-Tech Industry Development Institute (Jeju, South Korea). All plants used in this study were shredded and powdered. The powdered samples were then extracted with 70% (v/v) ethanol. After the sample was filtered through two layers of cheesecloth, the filtered cakes were extracted and filtered three more times to increase the extraction yield. These consecutive extracts were combined and then filtered through Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure, freeze-dried, and stored in a closed container until used.

Pathogens and antibacterial activity test. Two

<table>
<thead>
<tr>
<th>Plant names</th>
<th>Medicinal usage</th>
<th>Constituents</th>
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<tbody>
<tr>
<td>Mollugo pentaphylla</td>
<td>Anticancer, antitoxic and diuretic agent, in the treatment of cancer, enteritis, hepatitis, appendicitis, eye diseases, contusion, furunculosis</td>
<td>Mollugogenol-A (Rajasekaran et al., 1993)</td>
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<tr>
<td>Angelica anomal</td>
<td>Itching, poisonous effects, antiphlogistic agent, headache, megrim, toothache, ozena, stomach ache, hemorrhoids, vaginitis, rubella</td>
<td>Amomalín (Hata et al., 1966)</td>
</tr>
<tr>
<td>Matteuccia orientalis</td>
<td>Functional food</td>
<td>Matteuorien, matteuorienin, and matteuorienate A, B, and C (Basnet et al., 1995); matteuorienate A and B (Kadota et al., 1994)</td>
</tr>
<tr>
<td>Orixia japonica</td>
<td>Cough, arthritis, dysentery, swelling, malaria</td>
<td>Orixalone A, B, C, and D (Ito et al., 2004); orijanone, isopteleflorine and 3’-O-methylorixine (Noshita et al., 2001); japonine and eduline (Funayama et al., 2001); (−)-2-C-methyl-o-erythro-1,4-lactone (Ono et al., 2000)</td>
</tr>
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Gram-positive bacterial species, *Propionibacterium acnes* ATCC6919 and *Staphylococcus epidermidis* KCTC3958, were selected as test microorganisms because of their pathological capacity. *P. acnes* ATCC6919 was cultured at 37°C for 48 h in GAM broth (Nissui, Japan) under anaerobic conditions before the assay. *S. epidermidis* KCTC3958 was cultured at 37°C for 24 h with *Corynebacterium* media (casein peptone 10.0 g, yeast extract 5.0 g, glucose 5.0 g, NaCl 5.0 g per liter) before the assay. In vitro antimicrobial activity was determined by the broth microdilution method (Jorgensen et al., 1999) in 96-well microtiter plates. Two-fold dilutions of each extract were prepared in appropriate broth media at concentrations from 500 to 15.6 μg/ml. Each well was inoculated with 5 μl of bacterial suspension at a density of 10^7 CFU/ml. The microtiter plates were incubated at 37°C for 24 h. Microorganism growth was determined by measuring turbidity with a spectrophotometer (Power Wave, Biotech Inc., VT, USA) at 600 nm. The minimal inhibitory concentration is defined as the lowest broth concentration of 70% ethanol extract that resulted in no visible microorganism growth.

**Measurement of cytokine production.** Human monocyteic THP-1 cells (1 × 10^6) in serum-free medium were stimulated with 100 μg/ml (wt weight) of heat-killed *P. acnes*, alone or in combination with the indicated concentrations of *Mollugo pentaphylla*, *Angelica anomala*, *Matteuccia orientalis*, or *Orixa japonica* extracts, and were incubated for 48 h. The culture supernatants were then harvested. The concentrations of IL-8 and TNF-α in the culture supernatant were measured using Enzyme-linked Immunosorbent Assay (ELISA) kits (Genzyme, Minneapolis, MN, USA).

**Cytotoxicity assay.** HaCaT and human normal fibroblast cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Gibco) and penicillin-streptomycin at 37°C in a humidified 95% air/5% CO₂ 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 MTT assay in which 3-(4,5-di-methyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) is reduced to formazan. After HaCaT and normal fibroblast cells were incubated with various concentrations of *M. pentaphylla*, *A. anomala*, *M. orientalis*, or *O. japonica* extracts for 24 h at 37°C in 5% CO₂ 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).

**Results and Discussion**

We evaluated the effects of Jeju medicinal plants on antibacterial and anti-inflammatory activities against *P. acnes* and *S. epidermidis*. The 100 selected traditional Jeju herbal medicines were extracted with 70% ethanol, with extract yields ranging from 2.0 to 30.0% (data not shown). Among the medicinal plants, the extracts from *M. pentaphylla*, *A. anomala*, *M. orientalis*, and *O. japonica* effectively inhibited the growth of *P. acnes* and *S. epidermidis* in disc diffusion methods (zone of inhibition ≥15 mm). The subsequent experiments were conducted to determine MIC of the selected plant extracts. As seen in Table 2, four kinds of extracts exhibited notable antibacterial activity against *P. acnes* and *S. epidermidis*. The lowest MIC against *P. acnes* was produced by the *A. anomala* extracts (15.6 μg/ml). In the case of *S. epidermidis*, *M. orientalis* extracts showed the greatest antimicrobial effect. The MIC value was 31.2 μg/ml. Extracts from *M. pentaphylla* and *O. japonica* also showed moderate antibacterial activities against both pathogens.

Extracts of Jeju medicinal plants were examined for biological properties against inflammatory acne induced by *P. acnes* in terms of inhibitory effects of cytokine secretion. To determine the effect of plant extracts on the production of proinflammatory cytokines (TNF-α and IL-8), THP-1 cells were treated with four different

<table>
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<tr>
<th>Medicinal Plant</th>
<th>MIC (μg/ml) of the following bacteria</th>
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<tr>
<td></td>
<td><em>Propionibacterium acnes</em></td>
</tr>
<tr>
<td><em>Mollugo pentaphylla</em></td>
<td>250</td>
</tr>
<tr>
<td><em>Angelica anomala</em></td>
<td>15.6</td>
</tr>
<tr>
<td><em>Matteuccia orientalis</em></td>
<td>250</td>
</tr>
<tr>
<td><em>Orixa japonica</em></td>
<td>250</td>
</tr>
</tbody>
</table>

The minimal inhibitory concentration is defined as the lowest broth concentration of 70% ethanol extract that resulted in no visible microorganism growth.
extracts with the concentrations of 10, 50, and 100 μg/ml in the presence of heat-killed P. acnes (100 μg/ml; wet weight) for 48 h, and the cytokine levels were measured in the culture media. As shown in Fig. 1, cells treated with heat-killed bacteria showed an increase of TNF-α and IL-8 secretion. However, co-culture of cells with heat-killed P. acnes and plant extracts significantly suppressed the production of proinflammatory cytokines. The reduction of cytokine secretion was in a dose-dependent manner.

However, it is possible that the reduction of proinflammatory cytokines was caused by cytotoxic effects of the extracts. To determine whether the plant extracts induced cytotoxicity, we performed MTT assays in THP-1 cells. Extracts from M. pentaphylla, A. anomala, M. orientalis, and O. japonica had low cytotoxic effects at 100 μg/ml (data not shown). In order to apply the plant extracts for topical agents, the plant extracts

![Graph A](image)

![Graph B](image)

Fig. 1. Extracts of M. pentaphylla, A. anomala, M. orientalis, and O. japonica inhibit P. acnes-induced secretion of proinflammatory cytokines.

Dose-dependent effect of M. pentaphylla, A. anomala, M. orientalis, and O. japonica treatment on P. acnes-induced IL-8 (A) or TNF-α (B) release. THP-1 cells were stimulated with or without heat-killed P. acnes, and the supernatants were harvested. The secreted IL-8 (A) and TNF-α (B) in the culture supernatant were measured. Data are expressed as mean ± S.E.M. *p<0.05 vs. P. acnes alone. (+), treatment of heat-killed P. acnes; (−), no treatment of heat-killed P. acnes.
should not induce the cytotoxic effects on human skin cells when applied as a therapeutic agent for acne. We examined the cytotoxic effects of these four medicinal plants on the cultured cells, human dermal fibroblasts and keratinocyte HaCat cells. As shown in Fig. 2, >80% of cells were viable at 100 μg/ml of plant extracts (Fig. 2), suggesting relatively low cytotoxicity.

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, extracts of *M. pentaphylla*, *A. anomala*, *M. orientalis*, and *O. japonica* have relatively low cytotoxic effects suggesting the possibility of introducing them as safe topical therapeutic agents for acne.

Based on our results, we conclude that extracts of *M. pentaphylla*, *A. anomala*, *M. orientalis*, and *O. japonica* can be used for therapeutic agents of acne.

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**Fig. 2.** Cytotoxicity of four medicinal plants against human dermal fibroblasts (A) and HaCaT cells (B). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and penicillin-streptomycin with the extracts of medicinal plants for 24 h. The viability of cells was determined by the MTT assay. The absorbance was measured at 570 nm using a spectrophotometer. The experiment was performed in triplicate. >80% of cells were viable at 100 μg/ml of plant extracts.
However, although the antimicrobial and anti-inflammatory effect of *M. pentaphylla*, *A. anomala*, *M. orientalis*, and *O. japonica* extracts against acne-inducing bacteria was identified, their action mechanisms was not determined. In particular, their possible inhibition of proinflammatory cytokines remains to be evaluated in further studies. Nuclear factor-κB has been reported to be involved in the maximal transcription of proinflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-8, which are thought to be important in the generation of acute inflammatory responses. Therefore, *M. pentaphylla*, *A. anomala*, *M. orientalis*, and *O. japonica* extracts may inhibit NF-κB activation induced by *P. acnes*.

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