Antibacterial Effects of Medicinal Plants from Jeju Island against Acne-inducing Bacteria

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As therapeutic agents for acne, antibiotics are typically employed to inhibit inflammation or kill bacteria [Guin *et al.*, 1979]. Triclosan, benzoyl peroxide, azelaic acid, retinoid, tetracycline, erythromycin, macrolide, and clindamycin are among these antibiotics [Breathnach *et al.*, 1984]. However, antibiotic resistance has become increasingly prevalent within the dermatologic setting. The development of antibiotic resistance is multifactorial, involving the specific nature of the relationship of bacteria to antibiotics, for example, how the antibacterial is used, host characteristics, and environmental factors. To overcome antibiotic resistance, medicinal plants have been studied as alternative treatments for diseases. Traditional herbal medicines provide an interesting, largely unexplored source for the development of new drugs. Indeed, the potential use of traditional herbal medicines as a basis for new skin-care cosmetics has been emphasized recently [Kiken and Cohen, 2002]. It is of great interest to know 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 activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*.

An ethnobotanical survey was carried out in Jeju Island of South Korea from July to October 2005. Freshly picked plant components were air-dried at room temperature for 2 weeks with no direct sunlight. Voucher specimens were identified by Dr. G. Kim (JeJu High-Tech Industry Development Institute) 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 subsequently shredded and powdered. The powdered samples were then extracted with 70% (v/v) ethanol. After the sample was filtered

Table 1. The minimal inhibitory concentration (MIC) of Jeju medicinal plants on acne-inducing pathogenic microorganisms

Medicinal plants	Susceptibility of bacteria to medicinal plant extracts	
	Propionibacterium acnes	*Staphylococcus epidermidis*
	MIC (μg/mL)*	MIC (μg/mL)
Morus alba (Root)	15.625	3.125
Morus alba (Fruit)	250	250
Morus alba (Stem & Leaf)	500	62.5
Kalopanax pictus (Stem)	1000	125
Albizzia julibrissin (Bark)	250	125
Siegesbeckia glabrescens (Stem & Leaf)	>1000	1000
Poncirus trifoliata (Fruit)	125	500
Phellodendron amurense (Bark)	15.625	62.5

*The minimal inhibitory concentration is defined as the lowest broth concentration of 70% ethanol extract that resulted in no visible microorganism growth.

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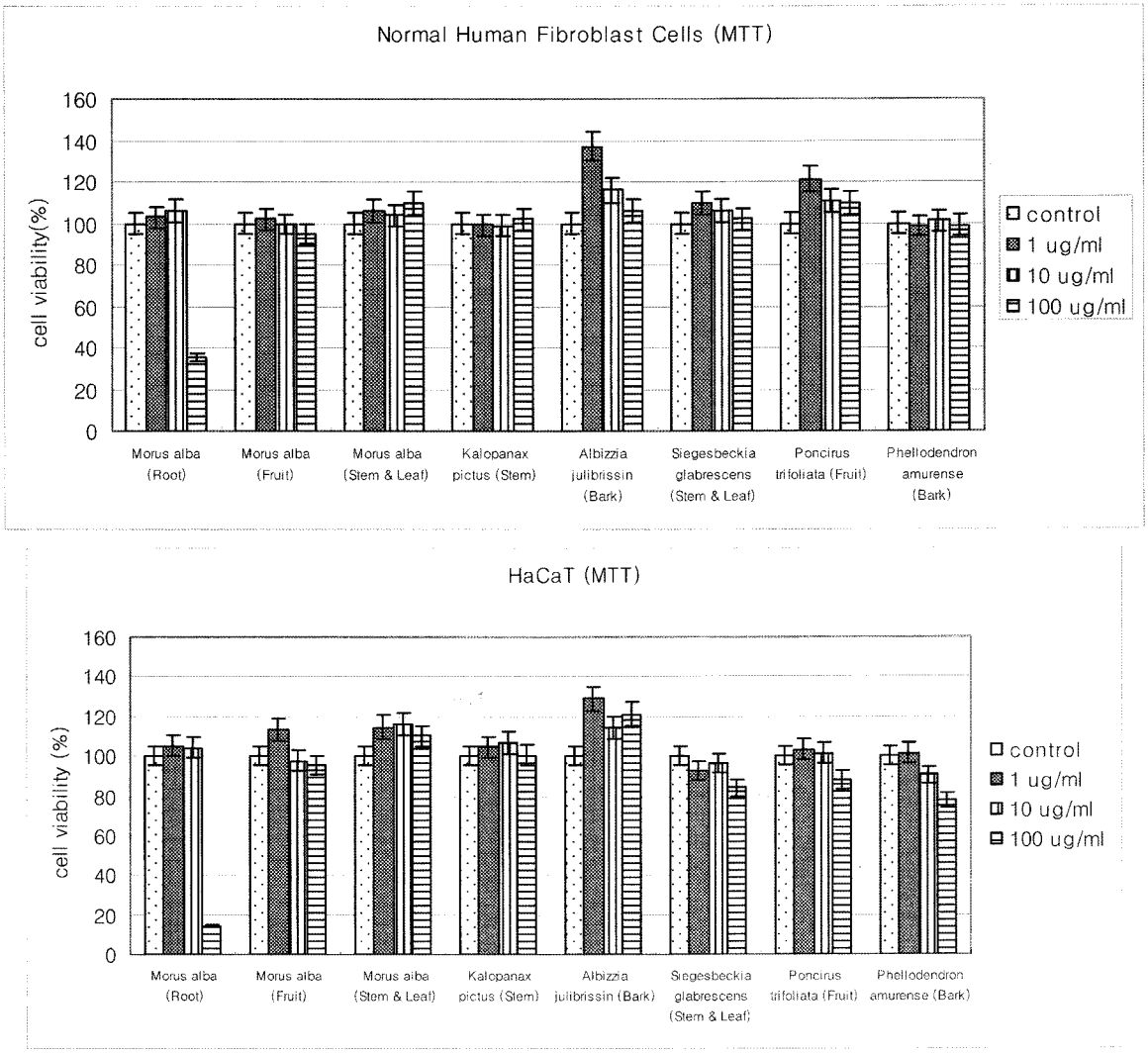

Fig. 1. Cytotoxicity of Jeju medicinal plants against normal human fibroblasts (A) and HaCaT cells (B). Human dermal fibroblast cells were cultured in DMEM (Hyclone) containing 10% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified 95% air: 5% CO_2 atmosphere. Cells were seeded on 24-well plates and sample treatment was initiated 24 h after seeding. General viability of cultured cells was determined by the reduction of MTT to formazan. The MTT assay was performed after incubation of normal fibroblast cells with various concentrations of plant extracts for 24 h at 37°C in 5% CO_2 atmosphere. MTT (1 mg/mL in phosphate-buffered saline) 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 using a spectrophotometer (Power Wave, Bio-tek Inc., VT, USA). The entire experiment was performed in triplicate, and the results were confirmed by three independent experiments.

through two layers of cheesecloth, the filtered cakes were extracted and filtered three more times to increase the extraction yield. All extracts were mixed together 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.

Two Gram-positive bacterial species that are involved in acne, *P. acnes* ATCC 6919 and *S. epidermidis* KCTC 3958, were selected as test microorganisms based on their pathological capacity. *P. acnes* ATCC 6919 was cultured at 37°C for 24 h in GAM broth (Nissui, Japan) under anaerobic conditions before the assay. *S. epidermidis* KCTC 3958 was cultured at 37°C for 24 h with *Corynebacterium* media before the assay. The minimal inhibitory concentration (MIC) of each test fraction was estimated by the broth dilution method. The MIC was taken as the lowest volatile fraction concentration that prevented visible bacterial growth after 24 h incubation at 37°C.

The *in vitro* antibacterial activity of Jeju medicinal plants against *P. acnes* and *S. epidermidis* was assessed by the presence or absence of inhibition zones and by MIC values. As seen in Table 1, the plant extracts exhibited notable antibacterial activity against *P. acnes*

and *S. epidermidis*. The lowest MIC values against *P. acnes* and *S. epidermidis* were produced by the *Morus alba* extract (15.6 μg/mL and 3.1 μg/mL, respectively). Extracts from *Phellodendron amurense, Albizzia julibrissin,* and *Poncirus trifoliate* also produced remarkably low MIC values against both pathogens. On the other hand, *Siegesbeckia glabrescens* exhibited very weak activities.

However, despite their promising antibacterial effects against these acne-inducing bacteria, the extracts could have cytotoxic effects on human skin cells when applied as a therapeutic agent for acne. If so, they would not be suitable therapeutic agents [Park *et al.*, 2004]. To examine the cytotoxic effects of selected medicinal plants, MTT assays were performed in both normal human fibroblasts and HaCaT cells [Mosmann, 1983]. All plant extracts displayed relatively low cytotoxicity, with cell viabilities above 80% at a concentration of 10 μg/mL (Fig. 1).

In conclusion, this work furthers our knowledge of the medicinal plants of Jeju Island and provides compelling evidence for the rational exploration of indigenous medicinal plants as a source of skin-care materials. Considering that most regional plants have not been investigated chemically or pharmacologically, they remain an untapped source of potential skin-care materials. Further investigations will focus on the *in vivo* assessment of the biological activity of these plant extracts and on the chemical identification of the major active components responsible for antibacterial activity in the efficacious extracts.

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