

Chemical Composition and Biological Activities of *Elsholtzia splendens* Essential Oil

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Propionibacterium acnes, a resident commensal bacterium that colonizes the lipid-rich environment of the pilosebaceous unit of the skin, produces chemotactic factors and proinflammatory molecules responsible for the inflammatory phase of acne. *P. acnes* secretes lipase and degrades sebum oils into free fatty acids, which are potent acne stimuli [Oprica *et al.*, 2005]. These free fatty acids stimulate the hair follicle, form the comedo, and finally induce inflammation [Downing *et al.*, 1986]. These bacteria also secrete leukocyte chemotactic factors for infiltrating the leukocytes in the hair follicle. These leukocytes stimulate and destroy the hair follicle wall. Subsequently, the contents of the hair follicle flow into the dermis [Webster *et al.*, 1980]. Therefore, *P. acnes* is considered to play an important role in the acne development by secreting the inflammatory factors.

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; IL-8, interleukin-8; MIC, minimal inhibitory concentration; NF- κ B, nuclear factor- κ B; PBS, phosphate-buffered saline; TMBZ, 3,3',5,5'-tetramethylbenzidine; TNF- α , tumor necrosis factor α .

As therapeutic agents for acne, antibiotics are usually employed to inhibit inflammation or kill bacteria [Breathnach *et al.*, 1984]. However, these antibiotics have been known to induce side effects. Several studies also reported on the possible side effects of tetracycline, erythromycin, macrolide, and clindamycin, such as appearance of resistant bacteria, organ damage, and immunohypersensitivity if these drugs are taken for a long period. Therefore, many investigators are interested in developing therapeutic agents for acne that have high antibacterial activity with no side effects [Park *et al.*, 2004; Tan, 2003].

The genus *Elsholtzia* is an ornamental, generally aromatic, herb that belongs to the Labiatae family. In Korea, five species are found, and the aerial parts of these plants have been used as an ingredient of folk remedies for cough, inducing sweat, blood circulation, rheumatism, inflammation, headache, and diuretics [Kim *et al.*, 2003].

Recently, the essential oil of *Elsholtzia splendens* has been found to possess several medicinal functions including an antioxidant effect [Jeong *et al.*, 2005] and an antimicrobial activity against several microorganisms [Kobold *et al.*, 1987]. However, the anti-inflammatory effect of *E. splendens* has not yet been described. Therefore, based on the previously known chemical composition [Lee *et al.*, 2005] and antibacterial effects of *E. splendens* essential oil, in the present study, the possibility of its effectiveness for acne treatment was investigated. The essential oil of *E. splendens* was analyzed to determine its chemical components, and the antibacterial activity of the oil was tested against *P. acnes* and *Staphylococcus epidermidis*, both of which are involved in acne. In addition, anti-inflammation and cytotoxicity of the oil were investigated.

Table 1 shows the composition of the volatile fraction of the plant in terms of the components and the classes of the compounds. Nine compounds were identified from the *Elsholtzia splendens* flower, representing more than 93% of the volatile compounds. Two compounds representing more than 88% of the essential oil were identified as dehydroelsholtzia ketone (82.46%) and elsholtzia ketone (5.96%). Other chemical components included 1-methyl-2(1*H*)-pyridinone (2.45%), α -humulene (1.18%), trans-caryophyllene (0.6%), α -bourbonene (0.27%), β -myrcene (0.16%), (*E*)-farnesene (0.16%), and (*E*)-trans-bergamota-2,12-dien-14-ol (0.13%).

Although the antimicrobial activities of the essential oils extracted from many plants have been recognized, albeit empirically, for centuries, only recently have such properties been confirmed [Lee *et al.*, 2007]. Botanical

Table 1. Chemical composition (%) of *E. splendens* essential oil

	Retention Time (min)	Constituent	Peak area (%)
1	4.122	β -Myrcene (C ₁₀ H ₁₆)	0.16
2	9.639	Elsholtzia ketone (C ₁₀ H ₁₄ O ₂)	5.96
3	9.804	1-methyl-2(1H)-pyridinone (C ₆ H ₇ NO)	2.45
4	11.713	Dehydroelsholtzia ketone (C ₁₀ H ₁₂ O ₂)	82.46
5	12.406	α -Bourbonene (C ₁₅ H ₂₄)	0.27
6	12.810	trans-Caryophyllene (C ₁₅ H ₂₄)	0.60
7	13.204	α -Humulene (C ₁₅ H ₂₄)	1.18
8	13.797	(E)-trans-bergamota-2,12-dien-14-ol (C ₁₅ H ₂₄ O)	0.13
9	15.365	(E)-Farnesene (C ₁₅ H ₂₄)	0.16

An ethnobotanical survey was carried out on Jeju Island, Korea in October 2006. 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, Korea). The essential oil of *Elsholtzia splendens* was extracted by hydrodistillation as described by Simard *et al.* Briefly, approximately 1 kg of the fresh *E. splendens* stem was immersed in 3.5 L of distilled water in a 5-L three-neck flask. Steam distillation was carried out for 12 h at the atmospheric pressure. Gas chromatographic analyses were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a polar Supelcowax column (30 m \times 0.25 mm \times 0.25 μ m), an apolar DB-1HT column (30 m \times 0.25 mm), and a split-splitless injection port (split mode). The temperature was set at 40°C for 5 min, ramped to 210°C at 10°C/min, and held at 250°C for 28 min. The compounds were identified by their retention indices on both columns and by GC-MS using a Hewlett-Packard MSD 5972 mass spectrometer at 70 eV coupled to an HP 5890GC equipped with a DB-1HT column (30 m \times 0.32 mm \times 0.1 μ m). The retention indices and mass spectra of all compounds were compared with those in the literature.

source, provenance of plant, harvest time or development stage, extraction technique, use of fresh or dried plant material, test micro-organism(s), and antimicrobial methodology are all factors that influence the antimicrobial activity [Cosentino *et al.*, 1999; Janssen *et al.*, 1987] and must, therefore, be taken into account whenever antimicrobial assays are performed using these oils.

Two Gram-positive bacterial species, *P. acnes* ATCC 6919 and *S. epidermidis* KCTC 3958 were selected as test microorganisms according to their pathological capacities. *P. acnes* ATCC 6919 was cultured at 37°C for 24 h in GAM broth (Nissui, Tokyo, Japan) under anaerobic conditions, and *S. epidermidis* KCTC3958 was cultured at 37°C for 24 h in *Corynebacterium* medium before the assay.

The disk diffusion method was used to elucidate the antibacterial activity of *E. splendens* essential oil against *P. acnes*. The MIC was taken as the lowest volatile fraction concentration that prevented visible bacterial growth after 24 h incubation at 37°C. *E. splendens* essential oil was found to have a significant antibacterial activity against *P. acnes*. However, no antibacterial activity was detected against *S. epidermidis*. The antibacterial activities of *E. splendens* essential oil were further evaluated by determining the MIC using a two-fold serial dilution method. *E. splendens* essential oil inhibited the growth of *P. acnes* at 0.31 μ L/mL.

The main proinflammatory mediators induced by bacteria and their cell components are cytokines, primarily TNF- α , and (IL-8). Therefore, to investigate the anti-inflammatory effects of *E. splendens* essential oil, ELISA was performed on IL-8 and TNF- α in the THP-1 cells. *P. acnes*-induced production of TNF- α and IL-8 in the THP-1 cells decreased by the addition of the *E. splendens* essential oil (Fig. 1A and B). However, the cytotoxic effect of the oil could also decrease the production of the proinflammatory cytokines. Thus, the cytotoxic effect of the *Elsholtzia* oil on the THP-1 cells was investigated using the MTT assay method, and the results showed the oil had no cytotoxic effect at 0.05 μ L/mL (data not shown). Even though the oil showed promising antibacterial and anti-inflammatory effects against the acne-inducing bacteria, it cannot be clinically introduced as a therapeutic agent for acne if cytotoxic effects on the human skin cells are detected. Thus, the cytotoxic effects of *E. splendens* essential oil on the human dermal fibroblasts cells were examined. The essential oil showed no cytotoxicity in the fibroblast cells at the concentrations below 0.1 μ L/mL (Fig. 2), an indication that *E. splendens* essential oil may be introduced as a possible therapeutic agent for acne. However, although the antimicrobial and anti-inflammatory effects of *E. splendens* essential oil against the acne-inducing bacteria was identified, its mechanism of action was not determined. In particular, the possible inhibitory

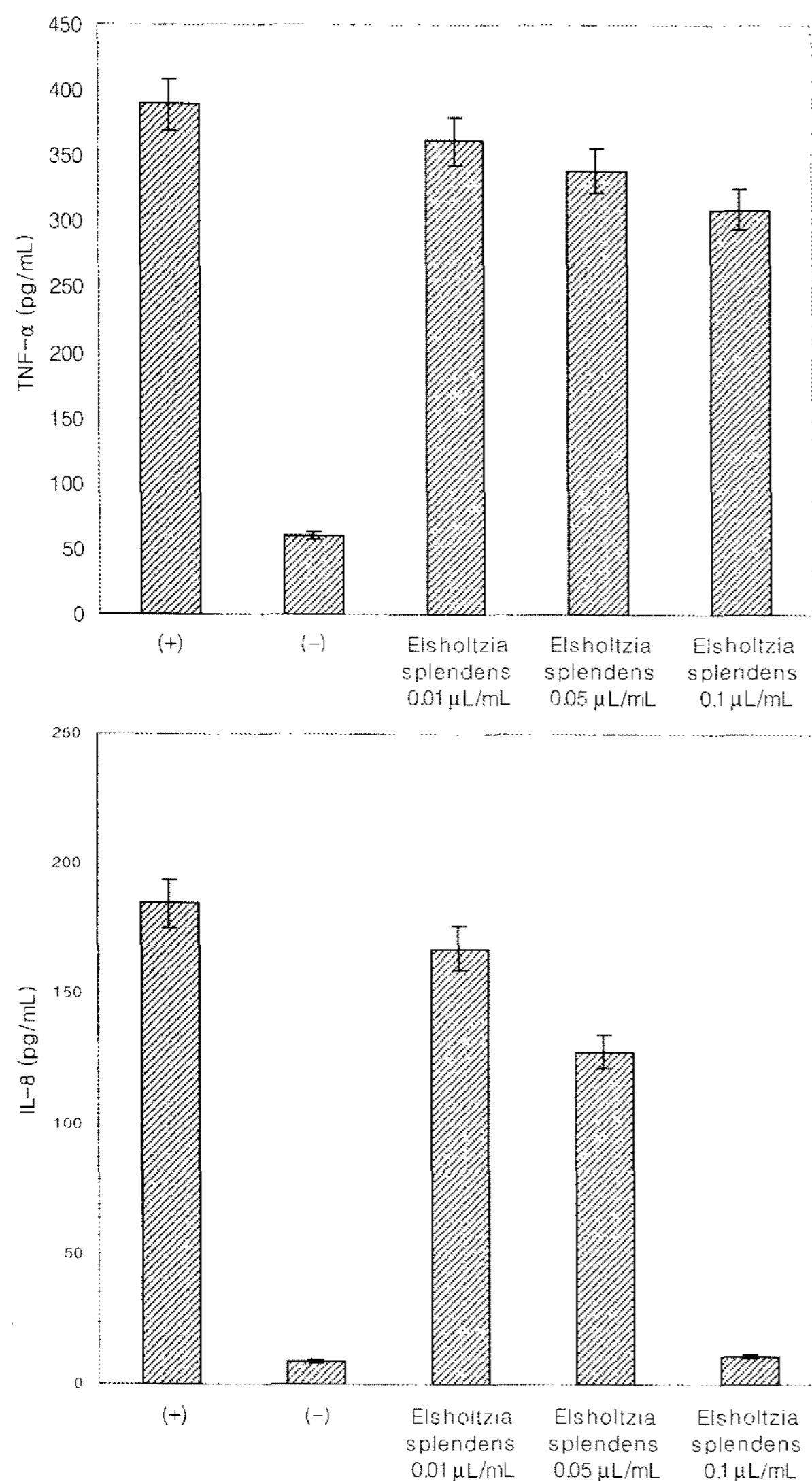


Fig. 1. Inhibition of *P. acnes*-induced secretion of proinflammatory cytokines, TNF- α (A) and IL-8 (B) by *E. splendens* essential oil. Dose-dependent effect of *E. splendens* treatment on the heat-killed *P. acnes*-induced TNF- α (A) or IL-8 (B) release. THP-1 cells were stimulated with or without *P. acnes*, and the supernatants were harvested for IL-8 and TNF- α measurements after 48 h. THP-1 cells were stimulated with or without *P. acnes*, and the supernatants were harvested after 48 h. Culture supernatants were added to 96 wells, and diluted biotinylated anti-IL-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 for 30 min at room temperature. After incubation, all wells were washed, and 3,3',5,5'-tetramethylbenzidine (TMBZ) substrate solution was added. The absorbance was read at 450 nm for 1214 min. Data are expressed as mean \pm SEM. * P <0.05 vs. *P. acnes* alone. (+); treatment of heat-killed *P. acnes*, (-); no treatment of heat-killed *P. acnes*.

mechanisms toward the proinflammatory cytokines remain to be evaluated in further studies. NF- κ B has been

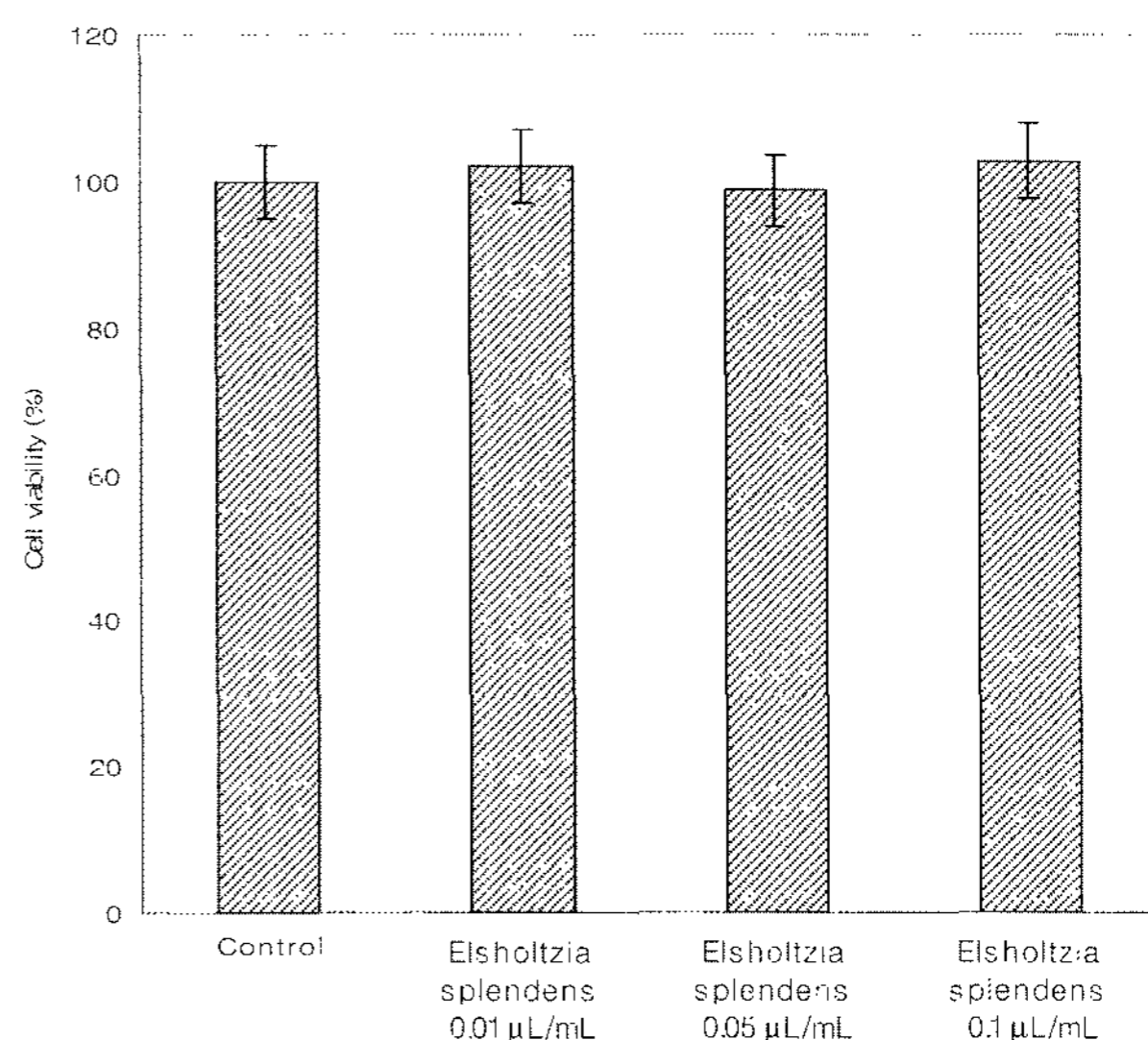


Fig. 2. Cytotoxicity of *E. splendens* essential oil against human dermal fibroblasts. Human dermal fibroblast cells (derived from neonatal foreskin) were acquired from the Amore-Pacific Corporation R&D Center, Korea. Human dermal fibroblast cells were cultured in Dulbecco's modified Eagle's medium containing 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-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide is reduced to formazan. After the dermal fibroblast cells were incubated with various concentrations of *E. splendens* essential oil 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 the medium. Cells were incubated at 37°C for 3 h, and dimethylsulfoxide was added to dissolve the formazan crystals. The absorbance was measured at 570 nm.

reported to be involved in the maximal transcription of several cytokines, including TNF- α , IL-1, IL-6, and IL-8, which are thought to be important in the generation of acute inflammatory responses [Yano *et al.*, 2008]. Therefore, it is possible that *E. splendens* essential oil may inhibit the NF- κ B activation induced by *P. acnes*.

In conclusion *E. splendens* essential oil has good antibacterial and anti-inflammatory effects against the acne-inducing bacteria, while inducing no cytotoxicity in human cell lines. Therefore, the *E. splendens* essential oil may be employed as an effective therapeutic agent to ameliorate the acne disease. To the best of our knowledge, this is the first report demonstrating the *in vitro* anti-inflammatory activity of *E. splendens* and providing a scientific basis for the cosmetic use of its essential oil.

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