# The essential oils of *Chamaecyparis obtusa* promote hair growth through the induction of vascular endothelial growth factor gene

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## ARTICLE INFO

## ABSTRACT

*Chamaecyparis obtusa* (*C. obtusa*) is a conifer in the cypress family *Cupressaceae*, native to northeast Asia. The essential oils of *C. obtusa* have antibacterial and antifungal effects and several products such as hygienic bands, aromatics, and shampoos contain these oils as a natural source of antimicrobial/antifungal agents. Interestingly, some consumers suffering from baldness and/or other forms of hair loss have reported a hair growth promoting effect of shampoos containing these oils. In the present study, the hair growth promoting effect of *C. obtusa* oils was elucidated in an animal model. *C. obtusa* oils promoted the early phase of hair growth in shaved mice. In addition, we examined the molecular effect of *C. obtusa* oils on the regulation of hair morphogenesis and hair growth using the human keratinocyte cell line HaCaT. In the current study of hair growth regulating genes, the expressions of *vascular endothelial growth factor (VEGF), transforming growth factor (TGFβ1), and keratinocyte growth factor(KGF)* have been analyzed by real-time PCR in HaCaT cells. The essential oils of *C. obtusa* were divided into seven fractions for treatment of HaCaT cells. *VEGF* transcripts were induced by fractions 6 and 7; however, *TGFβ1* and *KGF* mRNA levels were unchanged by *C. obtusa* oils or fractions. Fraction 7 was separated into seven sub-fractions and studied further. Sub-fractions E and D significantly increased *VEGF* and *KGF* gene expression without up-regulating the hair growth inhibition factor, *TGFβ1*. The components of the two sub-fractions were further analyzed by gas chromatography and mass spectrometry. Cuminol, eucarvone, and calamenene were common to these two sub-fractions, although the effects of these individual components were not determined. Taken together, these results suggest that *C. obtusa* oils promote hair growth in an animal model and a positive regulator of hair growth, *VEGF*, was induced by particular components of these oils.

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## 1. Introduction

The end product of hair follicle proliferation and differentiation is the hair shaft, which, together with surrounding root sheaths, is derived from epithelial cells [1]. The dermal papilla, a cluster of mesenchymal cells at the base of the follicle, plays an essential role in hair growth. In humans, the formation of hair follicles takes place during embryogenesis, and no new hair follicles form after birth [1]. However, the character of individual follicles can change drastically over time [1]. Thicker and darker hairs replace fine, lightly pigmented hairs in the beard at puberty. Conversely, thick scalp hairs convert into fine small hairs later in life [2]. The hair follicle is remodeled during cyclical periods of growth (anagen), regression (catagen), rest (telogen), and shedding

(exogen) [1,3]. A common disorder of hair growth is alopecia, a generic term for hair loss resulting in a diminution of visible hair [2]. Although hair disorders are not life threatening, their profound impact on social interactions and on patients' psychological well-being is undeniable.

*Chamaecyparis obtusa* (*C. obtusa*, commonly known as Hinoki) is a slow-growing tree with dark red-brown bark which grows to 35 m tall with a trunk up to 1 m in diameter. The leaves are scale-like, 2–4 mm long, blunt tipped (obtuse), green above, and green below with a white stomatal band at the base of each scale-leaf. We have previously reported on the antimicrobial and antifungal effects of *C. obtusa* oils [4]. The essential oils from *Pinus koraiensis* and *C. obtusa* inhibit the growth of gram-positive and -negative bacteria, as well as fungi [4]. In addition, Yang et al. reported that the essential oil of *C. obtusa* showed relatively strong antibacterial activities against gram-positive bacteria and some fungi [5].

Based on the antibacterial and antifungal effects and the fragrance of these essential oils, they have been used in several commercial products, including aromatics, deodorizing agents, hygienic bands, and personal care products i.e., shampoos. Interestingly, some consumers suffering from baldness and/or hair loss have reported that shampoos containing *C. obtusa* oils promoted hair growth. Therefore, in this study, we examined the hair growth promoting effect of *C. obtusa* essential oils *in vivo* using an animal model. In addition, the molecular mechanism of their effect(s) on promoting hair growth was further clarified *in vitro* using the human keratinocyte cell line HaCaT [6].

## 2. Materials and methods

### 2.1. Animal experiments

Male C57BL/6 mice (6-weeks-old, 5 mice per group) were obtained from KOATECH (Pyeongtaek, Gyeonggi, Korea). All animals were housed in polycarbonate cages, and acclimatized to an environmentally controlled room before experimentation (temperature, $23 \pm 2$ °C; relative humidity, $50 \pm 10\%$; frequent ventilation and 12 h light cycle). Experiments were performed with the approval of the Animal Ethics Committee at the College of Veterinary Medicine, Chungbuk National University. All animals were shaved using an animal clipper at 7 weeks of age, at which time all of the hair follicles were synchronized in the telogen stage [6]. Starting the following day (day 1), 0.2 ml of a 1% solution of *C. obtusa* extract in 40% ethanol was applied topically, on a daily basis, for 30 days. Ethanol (40%) was used as a vehicle in an animal experiment. Promotion of hair growth was evaluated by observing the skin color, which is indicative of the telogen-to anagen conversion [7]. The hair growth was calculated using the fallowing hair growth score: score 0, no hair growth observed; score 1, less than 30% growth observed; score 2, 30% to less than 50% growth observed; score 3, 50% to less than 70% growth observed; score 4, 70% to 100% growth observed [8].

### 2.2. Fractionation of C. obtusa oil

The essential oil was produced by water vapor distillation method. The volatile fraction was condensed, and used as crude essential oil. *C. obtusa* essential oils were fractionated by silica gel column chromatography in order to identify active components of the essential oil. The essential oil (240.40 g) was loaded on a silica gel column ($15 \times 29.5$ cm, #1.07734.9025, Merck, Darmstadt, Germany) and eluted with a mixture of hexane and ethyl acetate (20:1, v/v) at a flow rate of 20 ml/min. Fractions of 100 ml were collected in Erlenmeyer flasks (#A0205, Dong-Sung Science, Bucheon, Korea,). The total number of fractions collected was 527. Finally, after the above elution, the residue adsorbed to the silica gel was re-eluted with two column-bed volumes of ethyl acetate, and the eluate was labeled fraction 528. The fractions were pooled into seven groups according to composition, as visualized by TLC (Thin Layer Chromatography, plate #1.05715.0001, Merck) using 50% sulfuric acid and 50% hexane:ethyl acetate (8:1, v/v) as the developing solvent. After concentration with a rotary vacuum evaporator, the fractions were labeled 1 to 7.

The fraction 7 was further fractionated by silica gel column chromatography. Fraction 7 (5 ml) was loaded onto a silica gel column ($4.5 \times 31$ cm) and eluted with a mixture of hexane and ethyl acetate (10:1, v/v) at a flow rate of 20 ml/min. Fractions (250 ml) were collected in Erlenmeyer flasks; a total of 43 fractions were collected. The residue adsorbed to the silica gel was re-eluted with 1 l of ethyl acetate and labeled fraction 44. The original eluate was pooled into seven fractions according to composition, as visualized by TLC using 50% sulfuric acid and 50% hexane:ethyl acetate (8:1, v/v) as the developing solvent. After concentration with a rotary vacuum evaporator, the fractions were labeled A to G.

### 2.3. Gas chromatography and mass spectrometry analysis

The active fractions of the essential oils were analyzed by gas chromatography (GC)/mass spectrometry (MS) to identify the constituents of the active fractions. The GC (#HP 6890, Hewlett Packard, Santa Clara, USA) was equipped with a DB-5 MS column (30 m × 0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, USA), and the injector temperature and detector temperature were maintained at 250 °C and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 ml/min. The initial oven temperature was maintained at 70 °C for 10 min, and then the temperature was raised to 280 °C at a rate of 5 °C/min and finally held at that temperature for 10 min. Mass spectrometry (#HP 5973N, Hewlett Packard) used the EI mode, and the conditions were as follows: electron energy, 70 eV; scan range, 35–600 amu; source temperature, 230 °C. The chemical structure of each constituent was identified by comparing mass data with standard library data.

### 2.4. Cell culture

The human keratinocyte cell line HaCaT was kindly provided by the Department of Dermatology, School of Medicine, Chungnam National University, Daejeon, Republic of Korea. HaCaT cells were maintained in Dulbecco's Modified Eagle Media (DMEM, Gibco/Invitrogen, Co., Carlsbad, CA, USA) containing penicillin, streptomycin (Gibco/Invitrogen), and 10% fetal bovine serum (FBS; Gibco/Invitrogen). For treatment with *C. obtusa* oil or sub-fractions thereof, $0.3 \times 10^6$ HaCaT cells were seeded in 6-well plates 24 h before treatment and chemicals were then added to each well for 24 h.

## 2.5. Cytotoxicity assays

Cell viability was determined by the Cell Counting Kit-8 (CCK-8, Dojindo Lab., Tokyo, Japan). Cells were seeded at a density of 2500/well in DMEM containing 10% FBS in 96-well plates and incubated for 24 h. Cells were then cultured for 24 h with 100 μl of growth medium containing the indicated concentrations of *C. obtusa* oil or its fractions which are dissolved in dimethyl sulfoxide (DMSO) as a solvent. CCK-8 (10 μl) was added to each well of the plate and incubated for 3 h in a $CO_2$ incubator. Absorbance was measured at 450 nm using a microplate reader with a reference wavelength at 600 nm. The cell viability rates were calculated and presented as percentages of the vehicle treated with DMSO.

## 2.6. Quantification of mRNA by RT-PCR

Total cellular RNA was prepared using the TRIzol reagent (Invitrogen, Co.,) and the concentration of RNA was determined by absorbance at 260 nm. RT-PCR was performed as previously described [9]. Briefly, total RNA (1 μg) was reverse transcribed into first strand complementary DNA (cDNA) using M-MLV reverse transcriptase (Invitrogen, Co.) and a random primer (9-mer, TaKaRa Bio., Inc, Otsu, Shiga, Japan). Target genes, *vascular endothelial growth factor* (*VEGF*), *transforming growth factor-β1* (*TGFβ1*), *keratinocyte growth factor* (*KGF*), *insulin-like growth factor-1* (*IGF-1*), *hepatocyte growth factor* (*HGF*), and *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) as an internal control were amplified as previously described [10]. Real-time PCR reactions (20 μL) contained 10 μL TaqMan™ Universal PCR Master Mix (Applied Biosystems, Foster, CA, USA), 1 μL 20X Assays-on-Demand™ Gene Expression Assay Mix (Applied Biosystems *VEGF*, Hs00173626_m1; *TGFβ1*, Hs00171257_m1; *KGF*, Hs001735 65_m1; *IGF-1*, Hs00153126_m1; *HFG*, Hs00300159_m1; and *GAPDH*, Hs00266705_g1) and 5 μL cDNA. PCR amplification was conducted using a 7300 Real-Time PCR System (Applied Biosystems), with an initial denaturation at 50 °C for 2 min, followed by 90 °C for 10 min and then 40 amplification cycles of denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 1 min. Relative expression levels were determined using the RQ software (Applied Biosystems). The expression of target genes was normalized to that of *GAPDH*.

## 2.7. Data analysis

Data were analyzed by nonparametric one-way analysis-of-variance using the Kruskal Wallis test, followed by Dunnett's test for multiple comparisons. Data values were converted to ranks for these tests. All statistical analyses were performed with SPSS for Windows (SPSS Inco, Chicago, IL, USA). *P* < 0.05 was considered statistically significant.

## 3. Results

### 3.1. Effect of C. obtusa oil on hair growth

Mice were shaved 1 day before topical application of *C. obtusa* oil for 30 days. During the application period, differences in average body weight between treatment and non-treatment groups or abnormalities in the animals were not observed.

Every 10 days, hair growth promotion was evaluated by observing the skin color. As seen in Fig. 1, the back of the mice were hairless at day 0 (hair growth score: 0). The hair growth promotion of the oil treated mice (hair growth score: 2) was higher than that of vehicle treated group (hair growth score: 1) at 10 days (Fig. 1). However further improvement in growth promotion by the oil was not detected and the both groups' hair growth score marked 4 at 20 or 30 days.

### 3.2. Effect of the C. obtusa oil and its fractions on HaCaT cell viability

To elucidate the molecular mechanism of *C. obtusa* oil in promoting hair growth *in vivo*, this oil was separated into seven fractions and used to treat HaCaT cells, a human keratinocyte cell line. Several growth factors correlated with hair growth were detected in cells treated with *C. obtusa* oil and its extracts.

First, the effect of DMSO, the solvent for *C. obtusa* oil and its fractions, on HaCaT cell viability was tested. HaCaT cells were treated with different concentrations of DMSO for 24 h. Cell viability sharply decreased between 3.1 and 6.3% DMSO (Fig. 2A). Thus, a final concentration of 2% DMSO in the medium was used for the vehicle control and *C. obtusa* oil or oil fractions. Cells were treated for 24 h with *C. obtusa* oil or its fractional extracts dissolved in DMSO to calculate the maximum dosages. As seen in Fig. 2B, the total oil and fractions exhibited cytotoxic effects on HaCaT cells at concentrations higher than 0.1% in DMSO. However these toxicities were reduced at dosages of 0.01% in DMSO. Therefore, *C. obtusa* oil and its fractions were used at a final concentration of 0.005% in DMSO (0.0001% in the growth medium). Based on these results, HaCaT cell were treated with the maximum dosages of the oil and its fractions for 24 h.

### 3.3. Effect of the C. obtusa oil and its extracts on expression of growth factor genes

Several growth factors correlated with the hair growth cycle have been reported [3,11,12]. In the present experiments, we used real-time PCR to analyze the expression of these growth factors in response to *C. obtusa* oil or fractions thereof. We first examined the expression levels of five growth factors (*VEGF*, *TGFβ1*, *KGF*, *IGF*, and *HGF*) in untreated HaCaT cells using real-time PCR. The internal control gene (*GAPDH*), *VEGF*, *TGFβ1*, and *KGF* mRNA expression were detected; however two other factors examined, *IGF* and *HGF*, were below the detection limit of RT-PCR assay in HaCaT cells (data not shown).

*VEGF* mRNA was significantly induced by fractions 6 and 7; however, the other fractions and the total extract did not affect *VEGF* expression (Fig. 3). The expression of *TGFβ1* and *KGF* appear to be induced in 6 or 7 fraction-treated group, however no significant alteration was observed. These two genes were not affected by *C. obtusa* oil. Fraction 7, which induced the most *VEGF* expression, was divided into seven sub-fractions, A to G, which were used to treat HaCaT cells. *VEGF* expression was induced by fractions 6 and 7, as well as all of sub-fractions of the fraction 7 (A to G) (Fig. 4). Sub-fractions A, B, D, E, and F induced more than 8-fold increase in *VEGF* gene expression, compared to the vehicle treated group. In addition, sub-fractions A, D, and E induced *KGF* mRNA expression and sub-fractions B, F, and G significantly
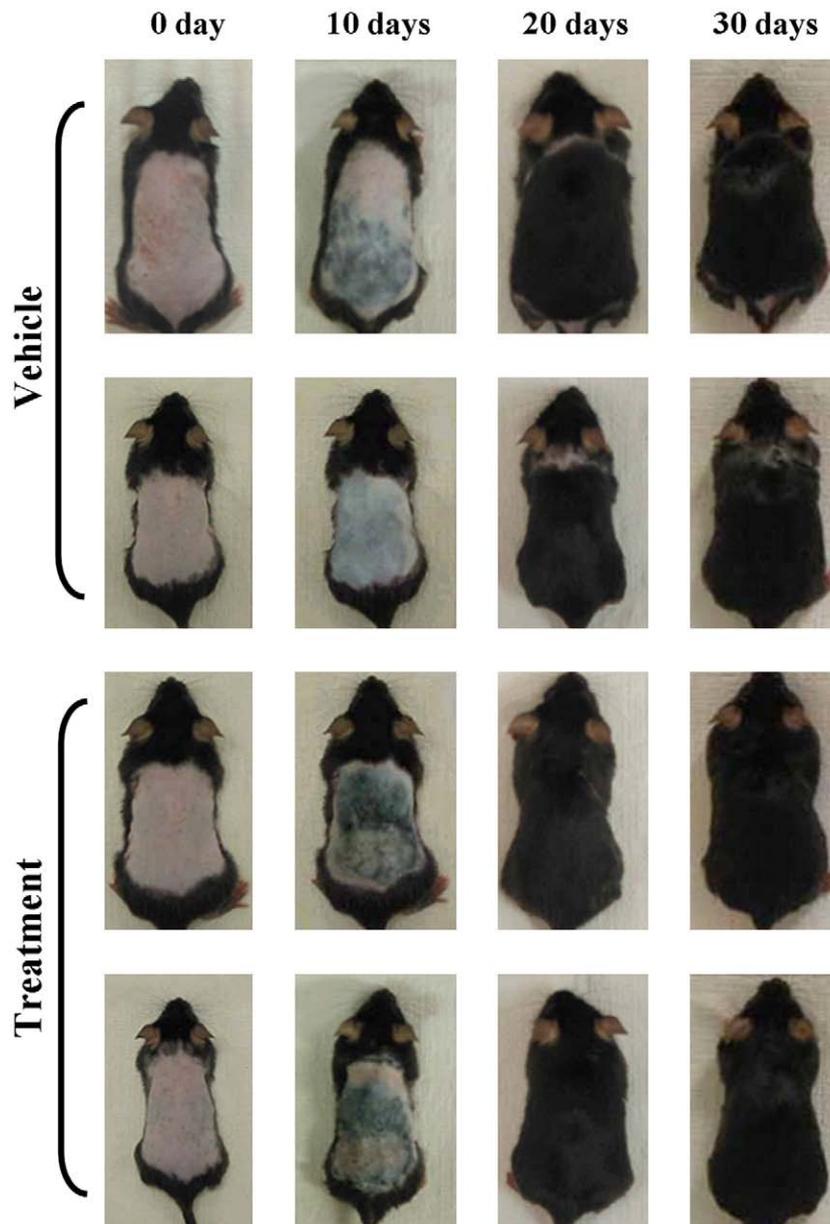
**Fig. 1.** Hair growth promoting effect of *C. obtusa* oils in mice. The backs of the mice were shaved and *C. obtusa* oils were applied topically according to the Materials and Methods. Photographs were taken every 10 days after shaving and hair growth on the back was compared between vehicle and oils treated groups.

increased *TGFβ1* mRNA expression. *VEGF* and *KGF* are stimulators of hair growth; however, *TGFβ1* has an inhibitory effect on hair growth [6]. These results suggest that certain factors in sub-fractions A, D and E up-regulate the hair growth promoting factors, *VEGF* and *KGF*, without increasing levels of the hair growth inhibiting factor, *TGFβ1*.

### 3.4. Qualitative analysis of the sub-fractions E and F of C. obtusa oil

To identify the compounds that induce *VEGF* and *KGF* transcription in HaCaT cells, sub-fractions A, D and E were analyzed by GC/MS. Sub-fraction A was contaminated with components of other sub-fractions, as indicated by the GC/MS analysis, and was not analyzed further. Sub-fraction D

consisted of 17 components (Table 1) and the major components were 1-methyladamantane (5.59%), cuminol (p-cymen-7-ol, 5.51%) and eucarvone (8.23%). In addition, sub-fraction E contained 10 components (Table 2) and 2-cyclopenten-1-one, 3,4-dimethyl-, 1,3-dimethyl-1-cyclohexene (4.1%), eucarvone (2.09%), and (-)-calamenene (1.02%) were major components of this sub-fraction. Interestingly, cuminol (p-cymen-7-ol), eucarvone, and (−)-calamenene were detected as common components in both sub-fractions.

### 4. Discussion

The essential oil of *C. obtusa* has been traditionally used in the treatment of urinary tract infections [4,5], and many
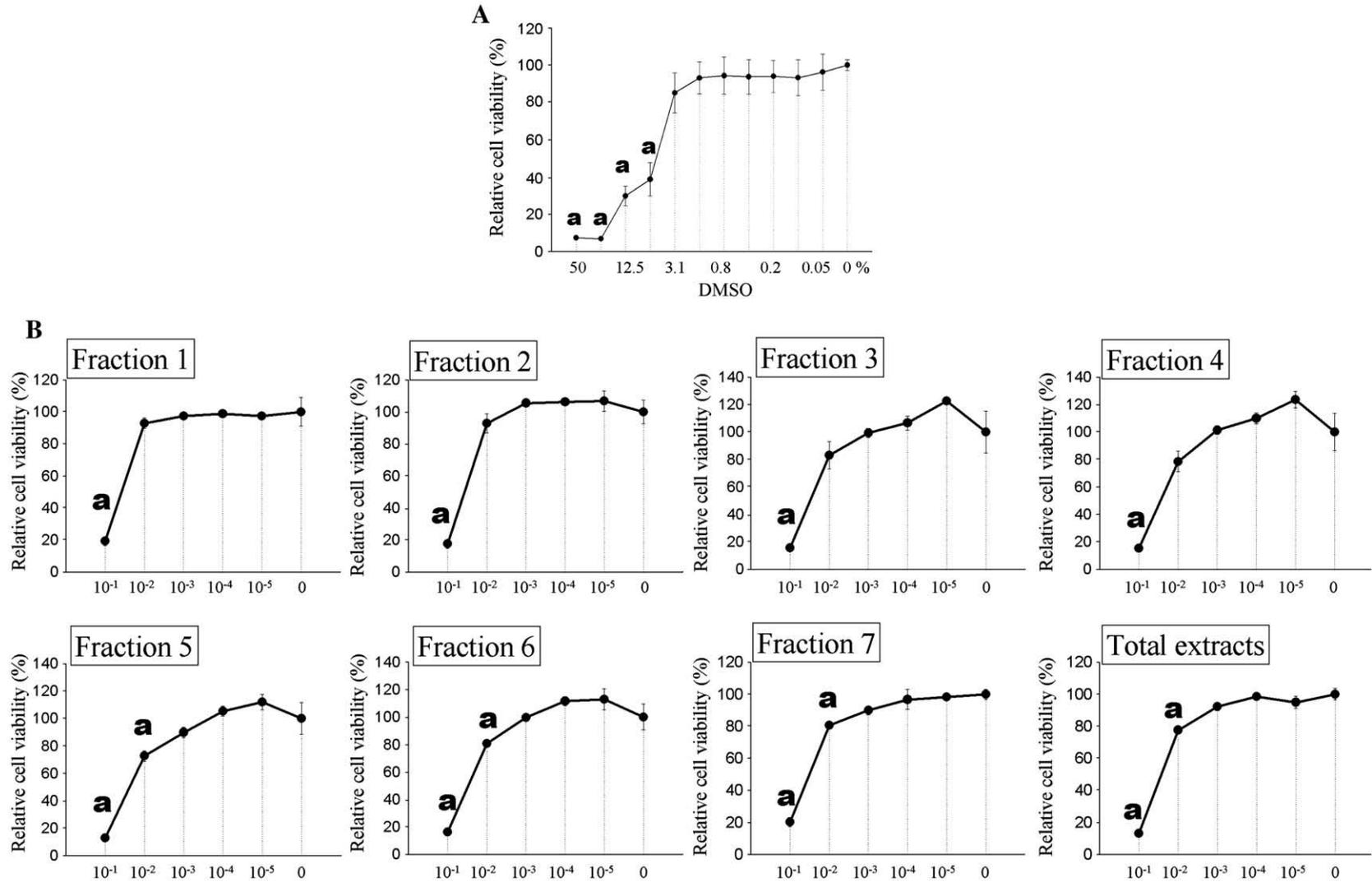
**Fig. 2.** Effect of DMSO, *C. obtusa* oils, and *C. obtusa* oil fractions on HaCaT cell viability. A, HaCaT cells were treated with increasing amounts of DMSO. The percent viability of the non-DMSO treated group was set to 100%. B, HaCaT cells were treated with decreasing concentrations of oils and oil fractions. The percent viability in the non-treated group was set to 100%. The graphs summarize the experimental analyses (means ± SDs of duplicate values from all samples). a, $P < 0.05$ *vs.* 0% DMSO for panel A and 0% oils or fractions for panel B.
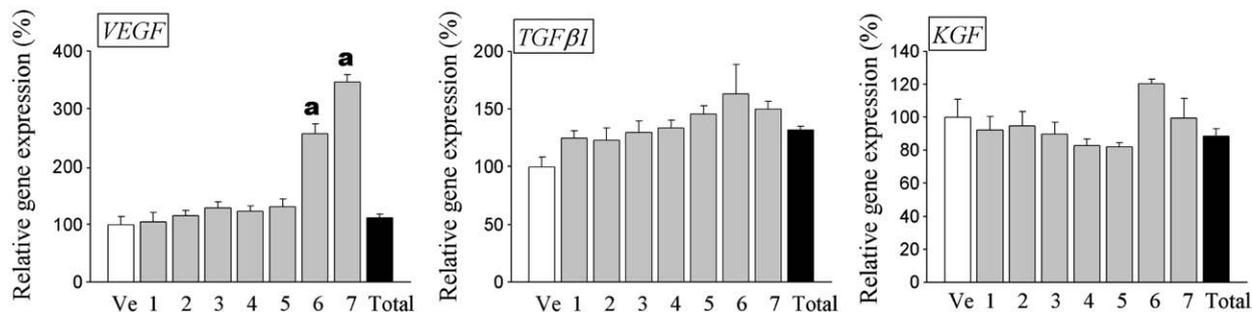
**Fig. 3.** The effect of *C. obtusa* oils and oil fractions on hair growth-related gene expression in HaCaT cells. HaCaT cells were treated with *C. obtusa* whole oil extract and seven oil fractions for 24 h and hair growth-related gene expression was analyzed using real-time PCR. The level of gene expression in the non-treated group was set to 100%. The graphs summarize the experimental analyses (means ± SDs of duplicate values from all samples). a, $P<0.05$ *vs.* vehicle group.

essential oils have been investigated for their antibacterial and antifungal properties [13]. Currently, several commercial products, such as shampoos, contain *C. obtusa* essential oil as a natural source of antimicrobial agent. Some consumers using these shampoos have reported an enhancement in hair growth rate. Therefore, the hair growth promoting effect of *C. obtusa* oil has been examined in the present study. Using an animal model, shaved mice were observed for 30 days with daily topical applications of essential oils or vehicle. At 10 days after shaving, hair growth in the oil treated mice was significantly enhanced, compared to the vehicle treated group. These data suggest that the essential oil of *C. obtusa* may mediate hair growth.

To clarify the molecular mechanism of *C. obtusa* in promoting hair growth, we measured the expression of several growth factors, *VEGF, KGF,* and *TGFβ1* in HaCaT cells. These growth factors are involved in the regulation of hair morphogenesis and hair growth [3]. Two fractions (6 and 7) significantly induced *VEGF* mRNA expression, but transcript levels of the other factors, *KGF* and *TGFb1*, were unchanged by treatment with the essential oil of *C. obtusa* and/or its fractions. It was further separated fraction 7, the most potent inducer of *VEGF* gene expression, into seven sub-fractions (A to G). All of the sub-fractions showed significantly higher *VEGF* gene expression than a vehicle treatment and did not induce cytotoxicity implicating components of fraction 7 in

the up-regulation of *VEGF* transcription. Although all of the sub-fractions increased *VEGF* expression in HaCaT cells, sub-fractions D and E were selected as candidates for a *VEGF* gene inducer, because sub-fractions B, F, and G also induced the hair growth inhibitor, *TGFβ1*. Sub-fraction A was contaminated with components of other sub-fractions, as indicated by the GC/MS analysis, and was not analyzed further.

*VEGF* is a key factor in promoting angiogenesis, as well as influencing diverse cell functions including cell survival, proliferation, and the generation of nitric oxide and prostacyclin [14]. *VEGF* also plays a role in inflammatory and neoplastic diseases associated with neovascularization [15]. The perifollicular capillary network is coupled to the hair cycle, increasing during the anagen phase and then regressing during the catagen and telogen phases [16]. Capillary proliferation during the anagen phase is temporally and spatially associated with *VEGF* expression in the outer root sheath of murine hair follicles [17]. These observations suggest that the vascular network is associated with the hair growth cycle [17,18,19]. *VEGF* mRNA expression in normal human hair follicles varies during the hair cycle [6,20]. In the anagen phase, *VEGF* mRNA is expressed strongly in the dermal papillae, whereas *VEGF* transcripts decrease in the catagen and telogen phases, suggesting that *VEGF* is an autocrine growth factor for dermal papilla cells [20]. Transgenic overexpression of *VEGF* in the outer root sheath
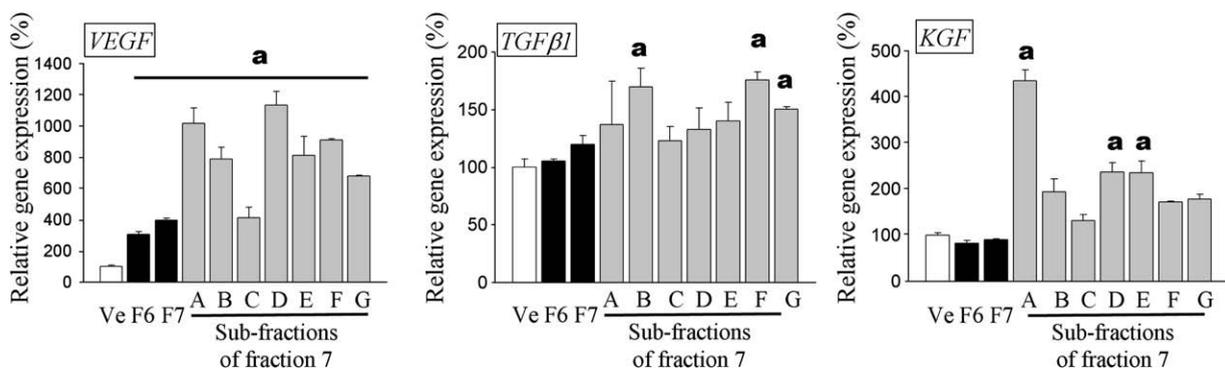


**Fig. 4.** Effect of fraction 7 sub-fractions on hair growth-related gene expression in HaCaT cells. Fraction 7 was separated into sub-fractions A–G. HaCaT cells were treated with these sub-fractions, as well as fractions 6 and 7 (F6 and F7), for 24 h and hair growth-related gene expression was analyzed using real-time PCR. The level of gene expression in the non-treated group was set to 100%. The graphs summarize the experimental analyses (means ± SDs of duplicate values from all samples). a, $P<0.05$ *vs.* vehicle group.

**Table 1**
Chemical compounds of sub-fraction D of *C. obtusa* oil identified by GC/MS.

| RT | Area (%) | CAS no. | MW | Library | Quality |
|---|---|---|---|---|---|
| 3.86 | 1.17 | 123-42-2 | 116.16 | Hydroxy-4-methyl-2-pentanone Pyraton | 83 |
| 17.11 | 0.59 | 4621-04-09 | 142.24 | Cyclohexanol, 4-(1-methylethyl)- | 83 |
| 18.54 | 0.4 | 122-00-9 | 134.18 | Ethanone, 1-(4-methylphenyl)-ρ-methyl-acetophenone | 97 |
| 18.67 | 5.59 | 768-91-2 | 150.26 | 1-Methyladamantane | 83 |
| 20.13 | 0.28 | 106-22-9 | 156.27 | β-Citronellol | 97 |
| 20.95 | 0.62 | 106-24-1 | 154.25 | Geraniol | 93 |
| 22.41 | 5.51 | 536-60-7 | 150.22 | p-Cymen-7-ol cuminol | 98 |
| 22.94 | 8.23 | 503-93-5 | 150.22 | Eucarvone | 91 |
| 23.54 | 0.73 | 22539-72-6 | 152.23 | p-Mentha-1,4-dien-7-ol | 90 |
| 24.73 | 0.51 | 5580-33-6 | 149.19 | 3,4-Dimethylbenzamide | 89 |
| 29 | 0.24 | 483-77-2 | 202.34 | (-)-Calamenene | 96 |
| 29.53 | 0.41 | 21391-99-1 | 200.32 | α-Calacorene | 93 |
| 29.68 | 0.41 | 639-99-6 | 222.37 | Elemol | 93 |
| 32.12 | 0.3 | 4630-07-3 | 204.35 | (+)-Valencene | 93 |
| 32.21 | 0.91 | 87-85-4 | 162.27 | Hexamethylbenzene mellitene | 95 |
| 46.65 | 0.26 | 103-23-1 | 370.57 | Di(2-ethylhexyl)adipate hexanedioic acid, bis(2-ethylhexyl) ester | 93 |
| 48.95 | 0.21 | 27554-26-3 | 390.56 | Diisooctyl phthalate | 91 |

RT, retention time; CAS, chemical abstracts service; area (%), relative amount of the sub-fraction; MW, molecular weight; quality, % similarity between library and component (only components with over 80% similarity are represented).

increases perifollicular vascularization and leads to accelerated hair growth following depilation and the growth of larger hairs [16]. Minoxidil, well known as a hair restorer, stimulates *VEGF* expression in cultured dermal papilla cells and, thus, *VEGF* may contribute to extension of the anagen phase in adrogenetic alopecia [21,22]. Certain chemicals in sub-fractions E and D of the *C. obtusa* essential oil stimulate *VEGF* transcription in keratinocytes and this action may contribute to the hair growth promoting effect in animal models.

Sub-fractions D and E were selected for further analysis, since they induced *VEGF* transcription in HaCaT cells. The components of these sub-fractions were analyzed using GC/MS. Sub-fraction D consisted primarily of 17 components and 10 major components were identified in sub-fraction E. Cuminol, eucarvone, and calamenene were identified as components common to both sub-fractions. Cuminol has been reported as an antibacterial, antifungal, antioxidant, and anti-carcinogenic agent [23] and eucarvone has been studied as a permeation enhancer on human skin [24]. In addition, calamenene induces immune responses to cancer by enhancing the differentiation and functional maturation of human monocyte-derived dendritic cells *in vitro* [25] and possesses anti-gastric-ulcer and anti-inflammatory properties *in vivo* [26]. Although the effects of these compounds common to sub-fractions D and E on *VEGF* transcription were not individually elucidated, whether these compounds induce *VEGF* induction will be investigated further.

In summary, the essential oils of *C. obtusa*, which are known to have antibacterial and antifungal effects, also promote hair growth in an animal model. Some fractions and sub-fractions of *C. obtusa* oils resulted in an up-regulation of *VEGF* transcription, which is tightly linked with a factor of promoting angiogenesis and hair growth. Although the specific compounds in these sub-fractions which regulate *VEGF* gene expression were not determined in the present study, some compounds of *C. obtusa* oils may have positive effects on hair growth in humans and animals via up-regulation of *VEGF*.

**Table 2**
Chemical compounds of sub-fraction E of *C. obtusa* oil identified by GC/MS.

| RT | Area (%) | CAS no. | MW | Library | Quality |
|---|---|---|---|---|---|
| 10.88 | 0.43 | 99-87-6 | 134.22 | ρ-Cymene | 95 |
| 18.99 | 0.31 | 1197-01-9 | 150.22 | ρ-Cymene-8-ol | 91 |
| 22.36 | 0.48 | 536-60-7 | 150.22 | p-Cymen-7-ol cuminol | 98 |
| 22.59 | 4.1 | 30434-64-1 | 110.15 | 2-Cyclopenten-1-one, 3,4-dimethyl- | 87 |
| | | 2808-76-6 | 110.2 | 1,3-Dimethyl-1-cyclohexene | 83 |
| 22.88 | 2.09 | 503-93-5 | 150.22 | Eucarvone | 90 |
| 29.01 | 1.02 | 483-77-2 | 202.34 | (-)-Calamenene | 98 |
| 30.06 | 0.46 | 30364-38-6 | 170.27 | 1,1,6-Trimethyl-1,2-dihydronaphthalene | 86 |
| 32.74 | 0.59 | 483-78-3 | 198.3 | Cadalene | 99 |
| | | | | Cadalin | |
| | | 489-84-9 | 198.3 | Azulene, 1,4-dimethyl-7-(1-methylethyl)- | 97 |

RT, retention time; area (%), the relative amount of the sub-fraction; CAS, chemical abstracts service; MW, molecular weight; quality, % similarity between library and component (only components with over 80% similarity are represented).

of Labor (MOLAB) through the fostering project of the lab of Excellency.

# References

[1] Cotsarelis G, Millar SE. Towards a molecular understanding of hair loss and its treatment. Trends Mol Med 2001;7:293–301.

[2] Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med 1999;341: 491–7.

[3] Danilenko DM, Ring BD, Pierce GF. Growth factors and cytokines in hair follicle development and cycling: recent insights from animal models and the potentials for clinical therapy. Mol Med Today 1996;2:460–7.

[4] Hong EJ, Na KJ, Choi IG, Choi KC, Jeung EB. Antibacterial and antifungal effects of essential oils from coniferous trees. Biol Pharm Bull 2004;27:863–6.

[5] Yang JK, Choi MS, Seo WT, Rinker DL, Han SW, Cheong GW. Chemical composition and antimicrobial activity of Chamaecyparis obtusa leaf essential oil. Fitoterapia 2007;78:149–52.

[6] Rho SS, Park SJ, Hwang SL, Lee MH, Kim CD, Lee IH, et al. The hair growth promoting effect of Asiasari radix extract and its molecular regulation. J Dermatol Sci 2005;38:89–97.

[7] Muller-Rover S, Handjiski B, van der Veen C, Eichmuller S, Foitzik K, McKay IA, et al. A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages. J Invest Dermatol 2001;117:3–15.

[8] Matsuda H, Yamazaki M, Naruto S, Asanuma Y, Kubo M. Anti-androgenic and hair growth promoting activities of Lygodii spora (spore of Lygodium japonicum) I. Active constituents inhibiting testosterone 5alpha-reductase. Biol Pharm Bull 2002;25:622–6.

[9] Lee GS, Choi KC, Jeung EB. Glucocorticoids differentially regulate expression of duodenal and renal calbindin-D9k through glucocorticoid receptor-mediated pathway in mouse model. Am J Physiol Endocrinol Metab 2006;290:E299–307.

[10] Lee GS, Lee KY, Choi KC, Ryu YH, Paik SG, Oh GT, et al. Phenotype of a calbindin-D9k gene knockout is compensated for by the induction of other calcium transporter genes in a mouse model. J Bone Miner Res 2007;22: 1968–78.

[11] Ozeki M, Tabata Y. In vivo promoted growth of mice hair follicles by the controlled release of growth factors. Biomaterials 2003;24:2387–94.

[12] Fujie T, Katoh S, Oura H, Urano Y, Arase S. The chemotactic effect of a dermal papilla cell-derived factor on outer root sheath cells. J Dermatol Sci 2001;25:206–12.

[13] Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 2003;10:813–29.

[14] Zachary I, Gliki G. Signaling transduction mechanisms mediating biological actions of the vascular endothelial growth factor family. Cardiovasc Res 2001;49:568–81.

[15] Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/ vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146:1029–39.

[16] Messenger AG, Rundegren J. Minoxidil: mechanisms of action on hair growth. Br J Dermatol 2004;150:186–94.

[17] Yano K, Brown LF, Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. J Clin Invest 2001;107:409–17.

[18] Ellis RA, Moretti G. Vascular patterns associated with categen hair follicles in the human scalp. Ann N Y Acad Sci 1959;83:448–57.

[19] Stenn KS, Fernandez LA, Tirrell SJ. The angiogenic properties of the rat vibrissa hair follicle associate with the bulb. J Invest Dermatol 1988;90: 409–11.

[20] Lachgar S, Moukadiri H, Jonca F, Charveron M, Bouhaddioui N, Gall Y, et al. Vascular endothelial growth factor is an autocrine growth factor for hair dermal papilla cells. J Invest Dermatol 1996;106:17–23.

[21] Lachgar S, Charveron M, Gall Y, Bonafe JL. Minoxidil upregulates the expression of vascular endothelial growth factor in human hair dermal papilla cells. Br J Dermatol 1998;138:407–11.

[22] Li M, Marubayashi A, Nakaya Y, Fukui K, Arase S. Minoxidil-induced hair growth is mediated by adenosine in cultured dermal papilla cells: possible involvement of sulfonylurea receptor 2B as a target of minoxidil. J Invest Dermatol 2001;117:1594–600.

[23] Teissedre PL, Waterhouse AL. Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. J Agric Food Chem 2000;48:3801–5.

[24] Kang L, Poh AL, Fan SK, Ho PC, Chan YW, Chan SY. Reversible effects of permeation enhancers on human skin. Eur J Pharm Biopharm 2007;67: 149–55.

[25] Takei M, Umeyama A, Arihara S. T-cadinol and calamenene induce dendritic cells from human monocytes and drive Th1 polarization. Eur J Pharmacol 2006;537:190–9.

[26] Esteves I, Souza IR, Rodrigues M, Cardoso LG, Santos LS, Sertie JA, et al. Gastric antiulcer and anti-inflammatory activities of the essential oil from Casearia sylvestris Sw. J Ethnopharmacol 2005;101:191–6.