



ORIGINAL ARTICLE

Melandrium firmum Extract Promotes Hair Growth by Modulating 5α -Reductase Activity and Gene Expression in C57BL/6J Mice

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Background: In our preliminary study, we screened for their potential to inhibit 5α -reductase, and *Melandrium firmum* (MF) extract showed the most potent activity as confirmed by high-performance liquid chromatography (HPLC). **Objective:** This study aimed to investigate the effects of MF extract on 5α -reductase activity and its mechanisms of action in the prevention or treatment of androgenetic alopecia. **Methods:** HPLC was used to measure 5α -reductase activity. The hair growth-promoting effect of MF extract in the shaved dorsal skin of C57BL/6J mice was studied for 30 days. Hair follicles were examined by histological examination. Protein and mRNA levels of growth factors involved in hair growth were determined by western blotting, and reverse transcription-polymerase chain reaction (RT-PCR) and qPCR, respectively. Cell proliferation was measured by (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. **Results:** MF extract at 0.5 mg/ml showed 43.5% inhibition of 5α -reductase. MF extract pro-

moted hair growth by inducing anagen phase reflected by skin color, hair density, and the number and size of hair follicles. It not only reduces the expression of transforming growth factor-beta 1 (TGF- β 1) and Dickkopf-1 (DKK-1), but also markedly upregulated insulin-like growth factor 1 and keratinocyte growth factor in the dorsal dermal tissue. Ursolic acid, ecdysterone, and ergosterol peroxide were identified as active constituents by activity-guided fractionation to inhibit 5α -reductase. They decreased the gene expression of TGF- β 1 and DKK-1 in human hair dermal papilla cells. **Conclusion:** In summary, these findings indicate that MF extract might be a good drug candidate for hair growth promotion. (**Ann Dermatol** 31(5) 502~510, 2019)

-Keywords-

Growth factors, Hair follicle, *Melandrium firmum*, 5α -reductase

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INTRODUCTION

The approximately 0.2% ~ 2% of the world population suffer from androgenetic alopecia^{1,2}. Hair loss occurs due to psychological and physical stress, and dandruff^{3,4}. So far, minoxidil and finasteride was approved as a drug against for the hair loss⁵⁻⁷. Minoxidil is an anti-hypertensive, has been shown to stimulate hair growth in the treatment of vasodilatation on body⁸; finasteride was promoted hair growth in androgenetic alopecia (AGA) with male patients⁹. However, these drugs have unpredictable efficacy and side effects, leading to limited therapeutic use¹⁰. Therefore, effective AGA treatment agents should be further developed. Recently, many researchers have reported natural extracts that promote hair growth^{11,12}.

Melandrium firmum (MF) is a herbal plant used to treat gonorrhoe, anuria and breast cancer and widely distributed in Korea^{13,14}. However, its effect on hair growth has not been reported. The aim of this study was to investigate effects of MF on the prevention or treatment of AGA by selectively inhibiting 5 α -reductase activity and its action mechanisms. Our study demonstrated that topical MF extract promotes hair growth in C57BL/6J mice.

MATERIALS AND METHODS

Preparation of MF

Whole plants of MF were purchased from Deakwang, Chuncheon, and the plant identification was confirmed by Emeritus Professor Heung Jun Chi (Department of Pharmacy, Seoul National University, Seoul, Korea). The MF was extracted with water (1.0 kg : 10 L) for 3 times, and filtered and concentrated to yield (167.32 g), and then suspended in distilled water and partitioned with hexane, EtOAc, and *n*-BuOH to give hexane-soluble (33.12 g), EtOAc-soluble (19.89 g), and *n*-BuOH-soluble (43.39 g) fractions, respectively. Among in the hexane-soluble obtained: fraction 1 (2.3 g), fraction 2 (1.6 g), fraction 3 (3.6 g), fraction 4 (2.9 g), fraction 5 (3.1 g), and fraction 6 (2.6 g), respectively. Finally, ursolic acid, ecdysterone, and ergosterol peroxide were isolated as active principle compounds from fraction 6 by 5 α -reductase inhibition (Fig. 1).

5 α -reductase assay

The 5 α -reductase assay was performed as described previously¹⁵ with slight modifications. Inhibitory activity of 5 α -reductase was determined by two special reactions; first, the replace extract with 0.2 ml of 50% ethanol to complete the reaction (rxn); second, an enzyme blank (ctrl) is added with 5.0 ml of dichloromethane before adding

nicotinamide adenine dinucleotide phosphate. The peak area ratio (*r*) of testosterone/internal standard was used to calculate the percentage inhibition rate (%) by the following formula:

$$\% = inhibition = \frac{(r_{sample} - r_{rxn})}{(r_{ctrl} - r_{rxn})} \times 100$$

Animal experimental protocol

Five-week-old male C57BL/6J mice (n=6) were controlled temperature (23°C ± 2°C), humidity (50% ± 10%), and a 12-hour light-dark cycle, and then acclimatization for seven days. All mice were carefully removed from the dorsal areas (2 × 4 cm) with animal clipper containing wax-rosin mixture¹⁶ and MF extract was applied daily on the dorsal skin of mice on day 1 post depilation for 30 days. Animal study was conducted in accordance with the Hallym University protocol (Hallym-2012-70-1).

Histological analysis

The dorsal skin samples were fixed in 4% formalin and embedded in paraffin block, cut into 5 μ m sections, and stained with hematoxylin and eosin to confirm hair follicle. Three different cross-sectional areas and the number of hair follicles were calculated using an image analysis program (Image-Pro Plus ver. 6.0; Media Cybernetics, Silver Spring, MD, USA).

Cell culture and proliferation

The human hair dermal papilla cells (HHDPCs) were incubated in mesenchymal stem cell medium (5% fetal bovine serum, 1% penicillin-streptomycin, and 1% mesangial stem cell growth supplement) at 37°C in 5% CO₂. HHDPCs were plated at 5 × 10³ cells per well in 96-well plates, and incubated in the presence or absence of ursolic acid, ecd-

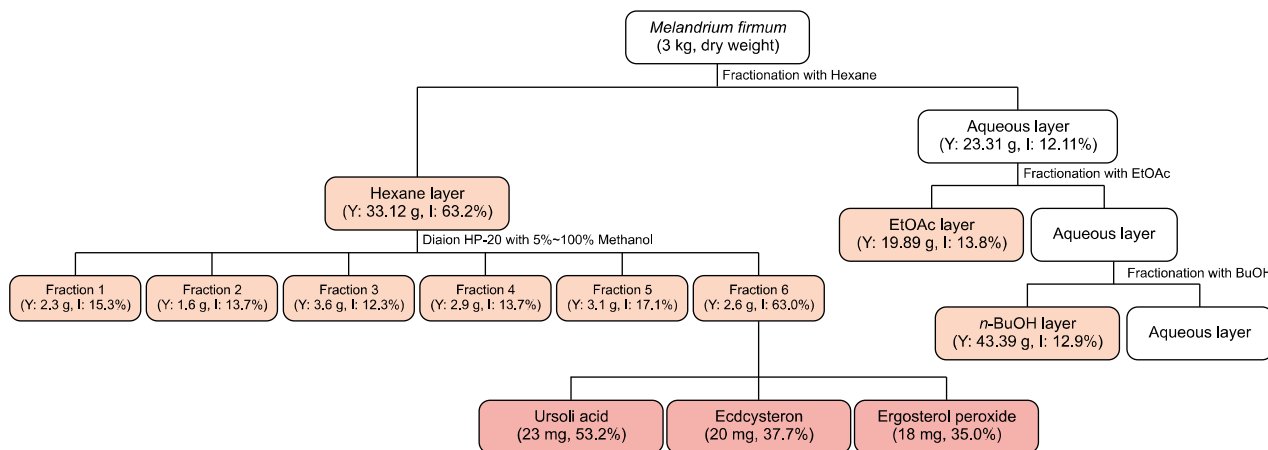


Fig. 1. Extraction and fractionation of *Melandrium firmum*. Y: yield, I: % inhibition.

cysterone, and ergosterol peroxide. After 24 and 48 hours of culturing HHDPCs, added 20 μ l of (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) solution to each well measured optical density at 550 nm using a microplate reader (Sensident Scan; Labsystems, Helsinki, Finland).

Reverse transcription-polymerase chain reaction (RT-PCR) and real-time polymerase chain reaction (PCR)

The total RNA was isolated from HHDPCs and dorsal dermal tissues and quantified using the NanoDrop-2000 (Thermo Fisher Scientific; Waltham, MA, USA). The cDNA synthesis was performed using a cDNA reverse transcription kit and real-time PCR was performed using the LightCycler real-time PCR System (Roche Applied Science, Indianapolis, IN, USA). The annealing temperatures and primers used for PCR reactions were listed in Table 1.

Western blot Analysis

The total protein (30 μ g) from HHDPCs and dorsal dermal tissue was lysed in Lysis buffer (Thermo Fisher Scientific) and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. After blotting, membranes were probed with transforming growth factor-beta 1 (TGF- β 1), Dickkopf-1 (DKK-1), insulin-like growth factor 1 (IGF-1), keratinocyte growth factor (KGF), and actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and incubation with secondary antibody for 1 hour at room temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$), immunoreactive proteins were visualized using an enhanced chemiluminescence substrate and quantified by densitometric analysis.

Statistical analysis

Student's unpaired t-test or one-way ANOVA were calculated with GraphPad Prism program (GraphPad Software Inc., San Diego, CA, USA). A p -value < 0.05 were considered statistically significant. Results are represented as the mean \pm standard error, and multiple comparisons were

carried.

RESULTS

Inhibitory effect of MF extract on 5 α -reductase activity

First, we investigated the effect of MF extract on 5 α -reductase activity. As shown in Fig. 2, 5 α -reductase activity was inhibited by 26.4%, 43.5%, and 57.1% in MF extract at concentrations of 0.1, 0.5, and 5 mg/ml, respectively (Fig. 2).

MF extract on hair regeneration in C57BL/6J mice

To investigate whether MF extract promotes hair growth, we induced the anagen phase of hair growth in C57BL/6J mice. After 30 days, MF extract induced telogen-to-anagen conversion earlier than vehicle treatment. During the experiment, visual scores were given weekly for hair growth (Fig. 3A). In the representative longitudinal section of histologic studies showed that MF extracts increased size of hair follicles and longer depth as compared with control

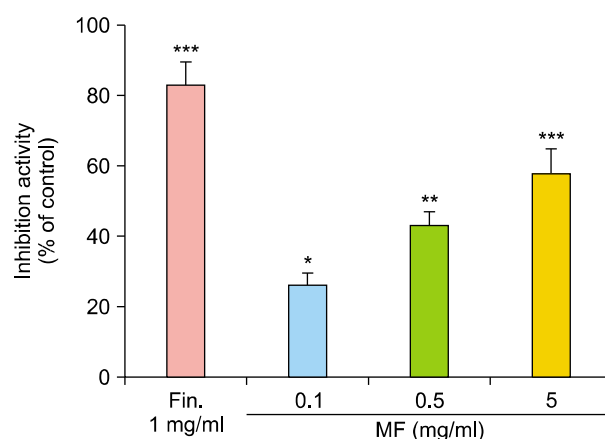


Fig. 2. Measurement of 5 α -reductase inhibitory activity. 5 α -reductase inhibitory activity was calculated using rat microsomes. Positive control was Finasteride. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. control. Fin.: finasteride, MF: *Melandrium firmum*.

Table 1. Primers sequence for RT-PCR and real-time PCR

Gene	Sequence of primer (5'→3')		Annealing temperature ($^{\circ}\text{C}$)
	Forward primer	Reverse primer	
TGF- β 1	AGACTTTTCCCCAGACCTCG	TGGGTGGTCTTGAATAGGGG	58
DKK-1	CCATTGACAACCTACCAGCCG	CTGCAGGCGAGACAGATTTG	58
IGF-1	TCAACAAGCCACAGGGTAT	ACTCGTGCAGAGCAAAGGAT	58
KGF	GACATGGATCCTGCCAATT	AATCCAACTGCCACTGTCC	50
Actin	GTCGTACCACTGGCATTGTG	GCCATCTCTGCTCAAAGTC	60

RT-PCR: reverse transcription-polymerase chain reaction, PCR: polymerase chain reaction, TGF- β 1: transforming growth factor-beta 1, DKK-1: Dickkopf-1, IGF-1: insulin-like growth factor 1, KGF: keratinocyte growth factor.

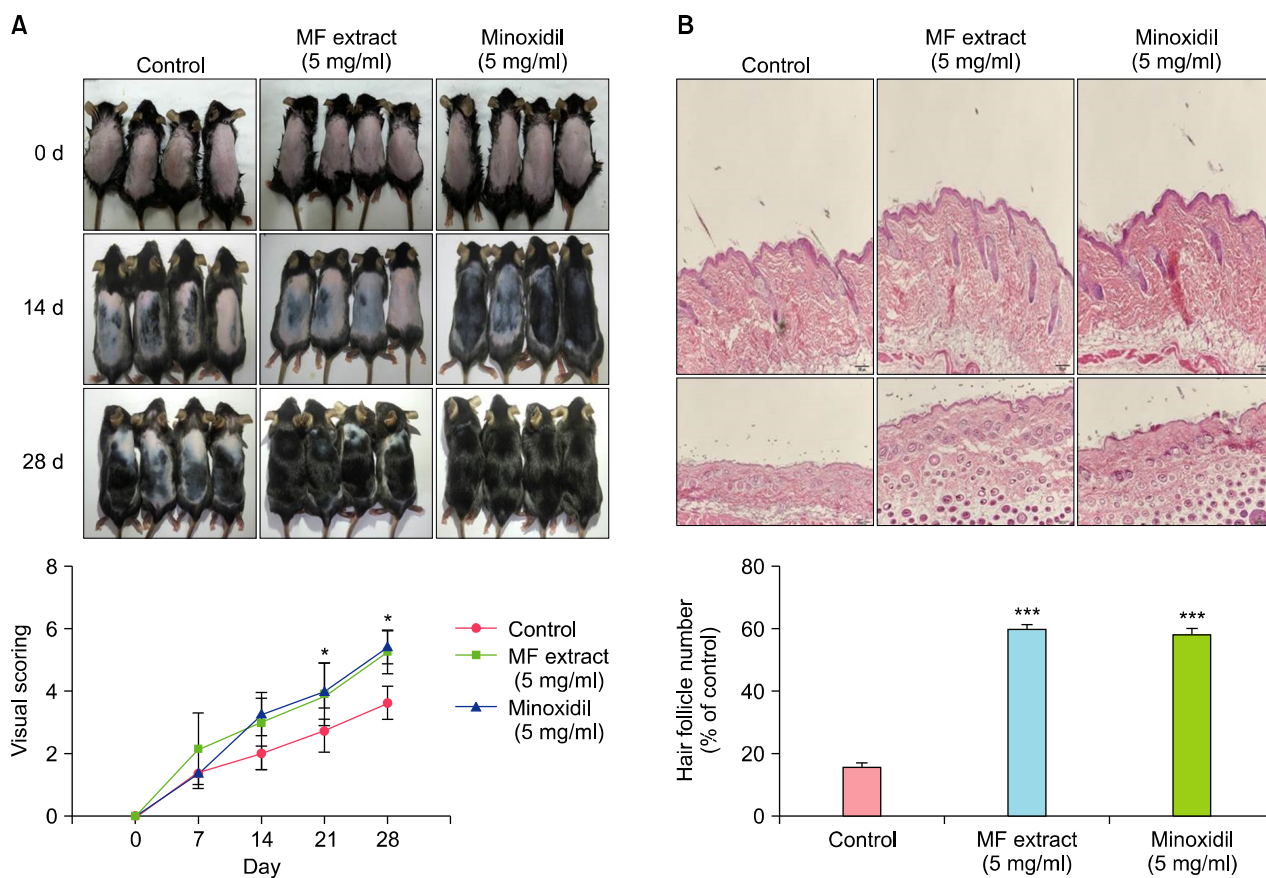


Fig. 3. Effect of *Melandrium firmum* (MF) extract on hair growth in C57BL/6J mice. (A) Comparison of dorsal skin colors and hair growth on days 0, 14, and 28 after depilation and the visual scoring of the hair growth-promoting effect of MF extract. (B) On the day the mice were executed, longitudinal sections of the dorsal skin were stained with hematoxylin and eosin and the number of hair follicles was determined based on morphology assessment at 200× magnification under bright-field microscopy. * $p < 0.05$ and *** $p < 0.001$ compared with control group.

group (Fig. 3B).

Effect of MF extract on growth factors in dorsal dermal tissues of C57BL/6J mice

In this study, we measured the gene and protein expression levels of TGF- β 1, DKK-1, IGF-1, KGF, and actin. As shown Fig. 4A and C, MF extract downregulated the expression of TGF- β 1 and DKK-1 compared to vehicle treatment. However, it upregulated the gene expression of IGF-1 and KGF (Fig. 4B, D).

Isolation of active components of the MF extract

To achieve a satisfactory separation of the multiple components in the MF extract, the 5 α -reductase activities of each fraction was examined using high-performance liquid chromatography (HPLC). The EtOH extract was fractionated into hexane, EtOAc, and *n*-BuOH extracts. The hexane extract significantly inhibited 5 α -reductase inhibition activity by 62.2%, whereas EtOAc and *n*-BuOH ex-

tracts showed no effect (Fig. 5A). We obtained six fractions from the hexane extract, and their 5 α -reductase inhibition activities were assessed. Fraction 6 of the hexane extract significantly inhibited 5 α -reductase activity by 63% (data not shown). Furthermore, we identified ursolic acid, ecdysterone, and ergosterol peroxide as the major active compounds in fraction 6. In addition, 5 α -reductase activity was inhibited by ursolic acid, ecdysterone, and ergosterol peroxide by 53.2%, 37.7%, and 35.0%, respectively (Fig. 5B).

Effect of ursolic acid, ecdysterone, and ergosterol peroxide on the proliferation and gene expression in HHDPCs

To assess the action mechanism responsible for the hair regeneration effects of MF extract in C57BL/6J mice, we investigated the effects of its major compounds on proliferation and gene expression in HHDPCs. Minoxidil (100 μ M) was used as a positive control. Proliferation of HHDPCs treated with MF extracts at 50 or 100 μ g/ml for

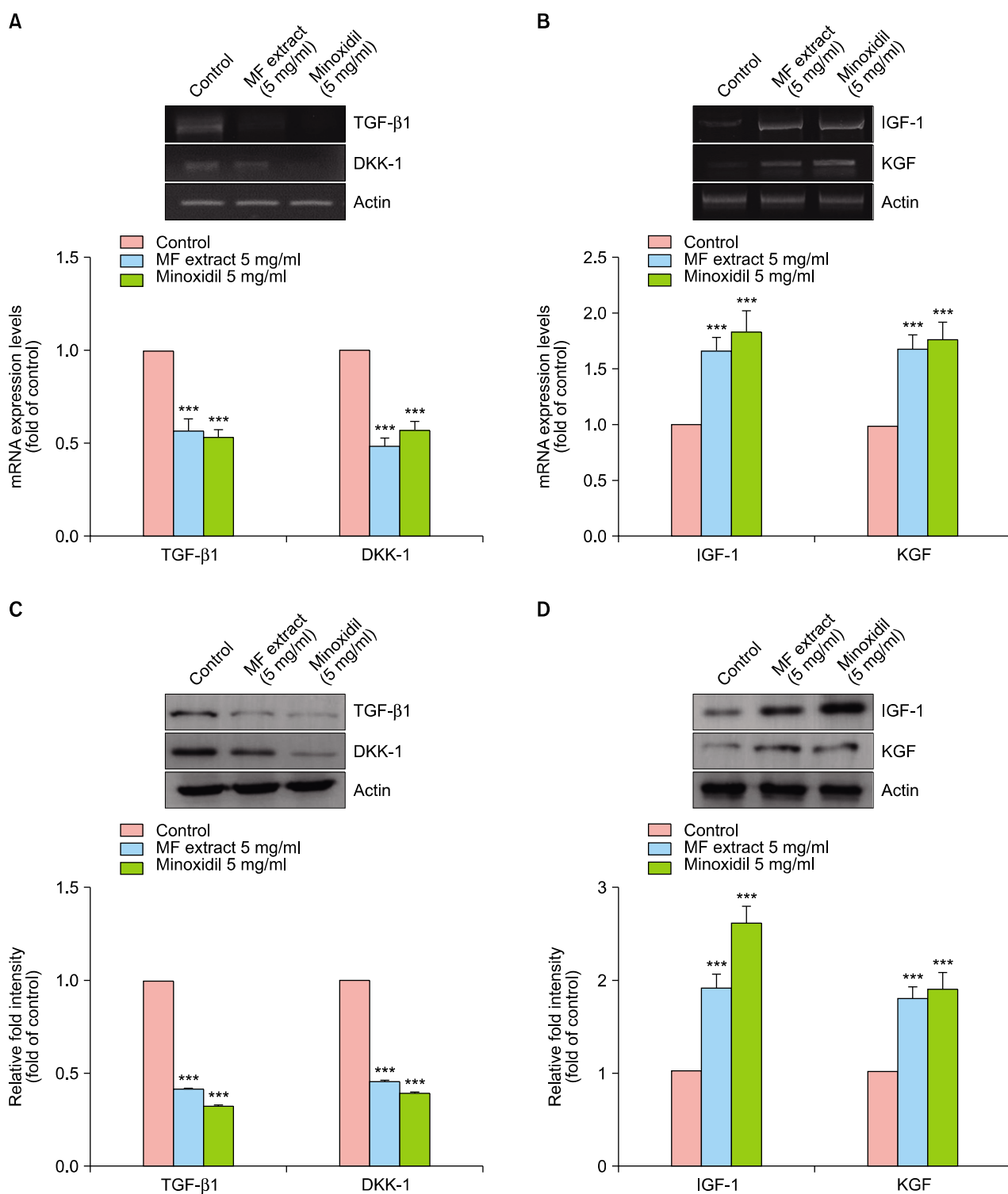


Fig. 4. Effect of *Melandrium firmum* (MF) extract on gene expressions in dorsal dermal tissues of C57BL/6J mice. Expression of TGF-β 1 and DKK-1 (A and C), IGF-1 and KGF (B and D) mRNA and protein levels were measured by RT-PCR and real-time PCR. Three independent experiments were carried out; *** $p < 0.001$ compared with control. TGF-β 1: transforming growth factor-beta 1, DKK-1: Dickkopf-1, IGF-1: insulin-like growth factor 1, KGF: keratinocyte growth factor, RT-PCR: reverse transcription-polymerase chain reaction, PCR: polymerase chain reaction.

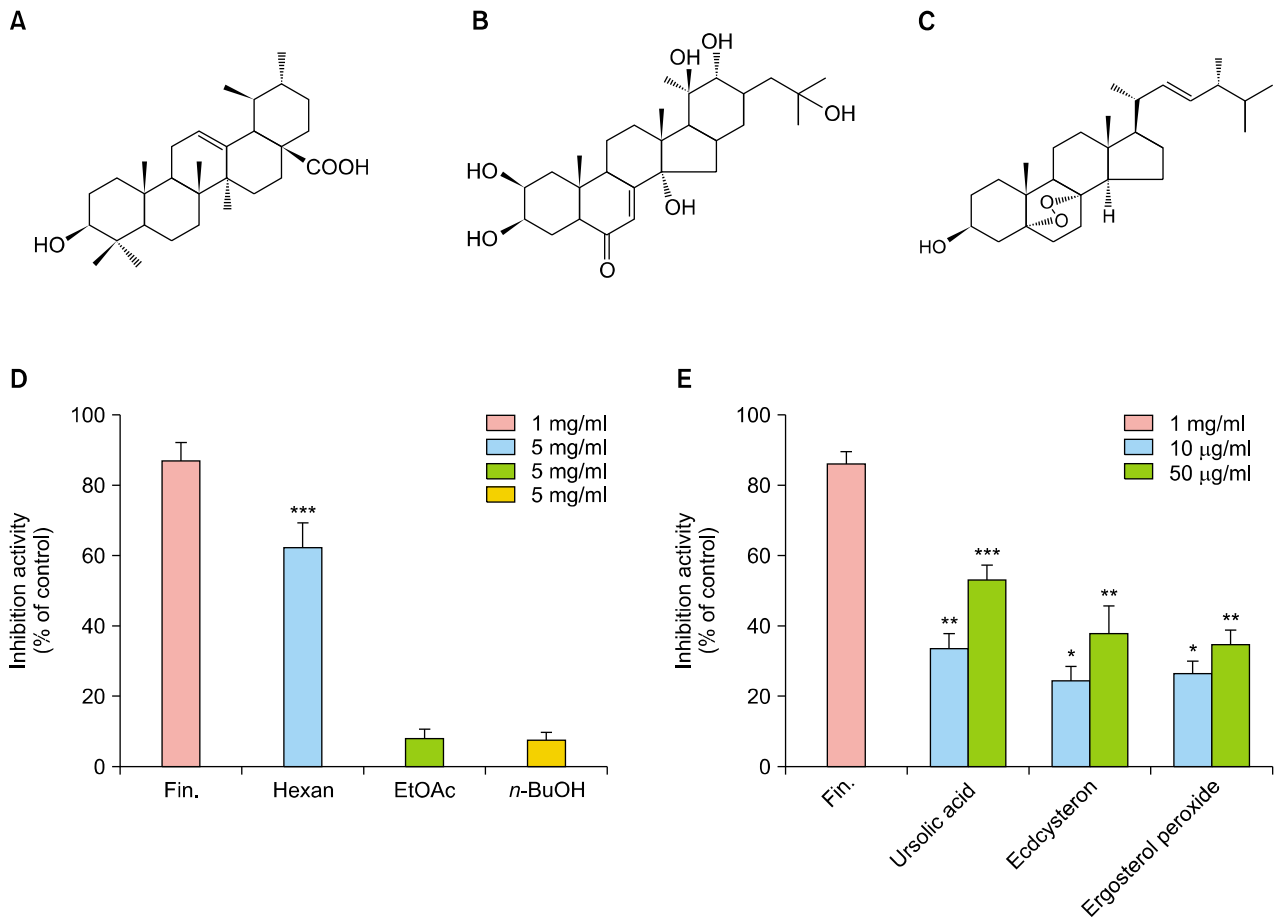


Fig. 5. Molecular structures of (A) ursolic acid; (B) ecdysterone; (C) ergosterol peroxide; (D) inhibition of 5 α -reductase as measured by high-performance liquid chromatography with various fractions of *Melandrium firmum* extract (5 mg/ml) and (E) its major active compounds (10 μ g/ml and 50 μ g/ml). * p <0.05, ** p <0.01, and *** p <0.001 compared with control. Fin.: finasteride.

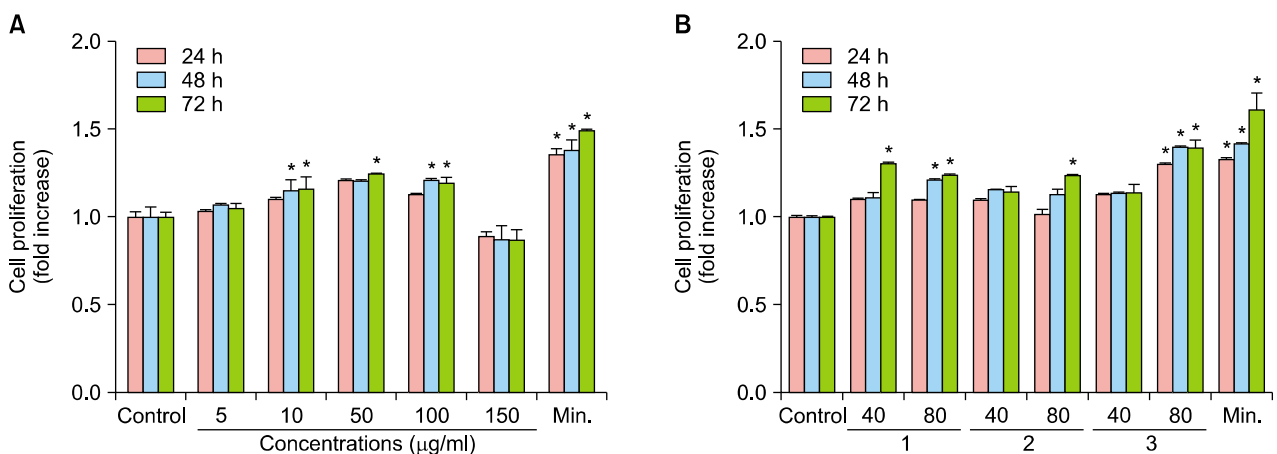


Fig. 6. Effect of *Melandrium firmum* (MF) extract and its major compounds on the proliferation of human hair dermal papilla cells (HHDPCs). (A) The cells were treated with various concentrations of MF extracts for 24, 48, and 72 hours. (B) HHDPCs were incubated with different concentrations of ursolic acid, ecdysterone, and ergosterol peroxide or minoxidil for 24, 48, and 72 hours. Lanes: 1, ursolic acid; 2, ecdysterone; 3, ergosterol peroxide. Three independent experiments were performed. * p <0.05 compared with control. Min.: minoxidil.

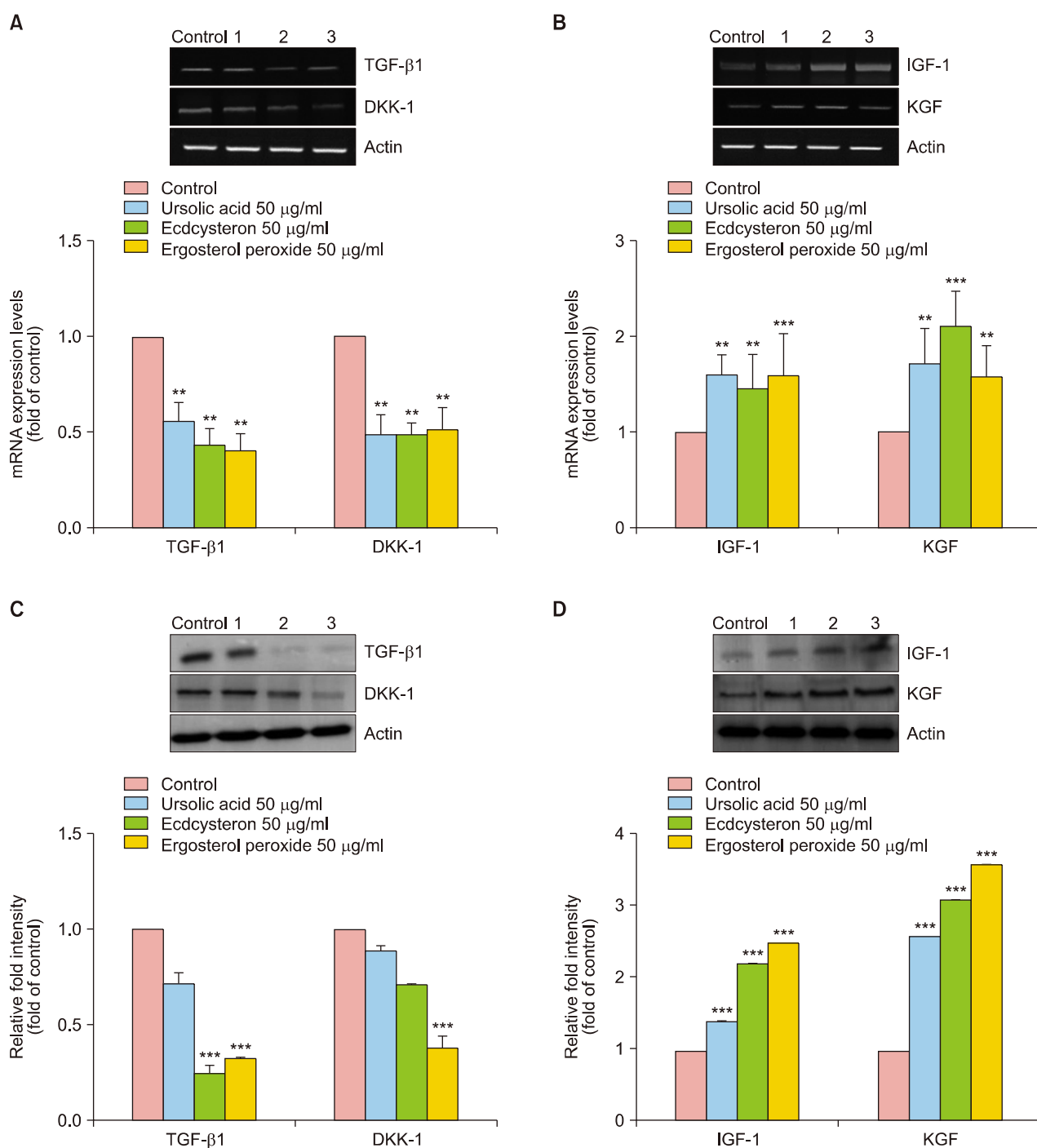


Fig. 7. Effect of ursolic acid, eccdysterone, and ergosterol peroxide on gene expression in human hair dermal palilla cells. Expression of TGF-β1 and DKK-1 (A and C), IGF-1 and KGF (C and D) mRNA and Protein were estimated by RT-PCR and real-time PCR. Lanes: 1, ursolic acid; 2, eccdysterone; 3, ergosterol peroxide. Three independent experiments were carried out; ** $p < 0.01$ and *** $p < 0.001$ compared with control. TGF-β1: transforming growth factor-beta 1, DKK-1: Dickkopf-1, IGF-1: insulin-like growth factor 1, KGF: keratinocyte growth factor, RT-PCR: reverse transcription-polymerase chain reaction, PCR: polymerase chain reaction.

72 hours was 121.8% and 119.0% higher, respectively, than that of the control group (Fig. 6A). Furthermore, ursolic acid, eccdysterone, and ergosterol peroxide also improved the proliferation of HHDPCs in time- and dose-

pendent manner (Fig. 6B). We also examined the expression of genes responsible for androgen signaling and hair cycle regulation in HHDPCs. As shown in Fig. 7A and C, the gene and protein expression TGF-β1 and DKK-1 were

lower in cells treated with ursolic acid, ecdysterone, and ergosterol peroxide than in control cells. However, these compounds induced the expression of IGF-1 and KGF, responsible for hair growth, in HDDPCs (Fig. 7B, D).

DISCUSSION

Hair is not only provides protective but also used to express personal beliefs or social position. Therefore, androgenic alopecia is considered a serious cosmetic problem in modern society. In our preliminary study, we screened 100 plants and found that MF extract showed the most effective 5 α -reductase inhibitory activity as confirmed by HPLC (data not shown). Based on this previous result, we explored whether MF extract promotes the growth of HDDPCs and hair regeneration in C57BL/6J mice. In the present study, our findings suggest that MF extract contributes to hair growth-promoting effect and regulated the expression of growth factors and inhibited 5 α -reductase activity.

The conversion of testosterone to dihydrotestosterone (DHT) by the enzyme 5 α -reductase, and DHT causes hair loss^{9,17}. Therefore, 5 α -reductase is considered as one of the most important targets for the development of hair loss drugs. In addition, TGF- β 1 and DKK-1 are important diffusion factors regulation the interaction between papilla-epithelium^{18,19}. The expression of the Wnt (wingless-type MMTV integration site family) ligand antagonist DKK-1 has been found to be up-regulated in response to DHT and reported to cause apoptosis in the bald scalp of patients with AGA^{20,21}. On the other hand, hair growth factor has also become one of the targets of hair loss treatment²². It is well known that IGF-1 and KGF is fundamental to stimulate the growth of hair follicles in HDDPCs^{23,24}. In the present study, MF extract inhibits 5 α -reductase activity as well as downregulates TGF- β 1 and DKK-1 gene and protein expression in C57BL/6J mice and stimulated increases in IGF-1 and KGF mRNA and protein levels in HDDPCs.

Based on these observations, MF extract represents a novel 5 α -reductase inhibitor and has been selected for further research, including screening of active component. In this study, we identified and isolated active components of MF, such as ursolic acid, ecdysterone, and ergosterol peroxide, guided by a 5 α -reductase inhibition assay using HPLC. Among them, ursolic acid showed an inhibition of 53.2% at 50 μ g/ml in accordance with previous results²⁵; it was obtained from fraction 6 of MF extract (hexane fraction). Interestingly, the inhibitory activity of ursolic acid was not greater than that of the MF extract and other components. Thus, synergistic effect, two other major compo-

nents isolated from the MF extract (hexane fraction), which are ecdysterone and ergosterol peroxide, also showed lower inhibitory activity than that of the MF extract. Therefore, the three compounds have synergistic effects and are ubiquitous phenomena in natural products²⁶. To understand their hair growth-promoting effects in HDDPCs, the androgen signaling pathway and key growth factor genes was studied. In *in vivo* study, we examined the expression of genes responsible for affecting the health of hair follicles and their growth in AGA. As shown in Fig. 7, the mRNA and protein expression of TGF- β 1 and DKK-1 were decreased by ursolic acid, ecdysterone, and ergosterol peroxide in HDDPCs when compared to control. However, these compounds markedly increased the expression of growth factors, such as IGF-1 and KGF, in HDDPCs. Therefore, our findings indicate that these bioactive compounds may be further studied for the treatment of AGA. Collectively, our results demonstrated that MF extract has a potent hair growth-promoting effect on C57BL/6J mice, suggests that MF extract and its active compounds may become a good candidate for promoting hair growth.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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REFERENCES

1. Kerscher M, Williams S, Dubertret L. Cosmetic dermatology

- and skin care. *Eur J Dermatol* 2007;17:180-182.
2. Paik JH, Yoon JB, Sim WY, Kim BS, Kim NI. The prevalence and types of androgenetic alopecia in Korean men and women. *Br J Dermatol* 2001;145:95-99.
 3. Kang JI, Kim SC, Han SC, Hong HJ, Jeon YJ, Kim B, et al. Hair-loss preventing effect of *Grateloupia elliptica*. *Biomol Ther (Seoul)* 2012;20:118-124.
 4. Ellis JA, Sinclair R, Harrap SB. Androgenetic alopecia: pathogenesis and potential for therapy. *Expert Rev Mol Med* 2002;4:1-11.
 5. Hadshiew IM, Foitzik K, Arck PC, Paus R. Burden of hair loss: stress and the underestimated psychosocial impact of telogen effluvium and androgenetic alopecia. *J Invest Dermatol* 2004;123:455-457.
 6. Burton JL, Marshall A. Hypertrichosis due to minoxidil. *Br J Dermatol* 1979;101:593-595.
 7. Kaufman KD, Olsen EA, Whiting D, Savin R, DeVillez R, Bergfeld W, et al. Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. *J Am Acad Dermatol* 1998;39:578-589.
 8. Shorter K, Farjo NP, Picksley SM, Randall VA. Human hair follicles contain two forms of ATP-sensitive potassium channels, only one of which is sensitive to minoxidil. *FASEB J* 2008;22:1725-1736.
 9. Murata K, Noguchi K, Kondo M, Onishi M, Watanabe N, Okamura K, et al. Promotion of hair growth by *Rosmarinus officinalis* leaf extract. *Phytother Res* 2013;27:212-217.
 10. Patel S, Sharma V, Chauhan NS, Thakur M, Dixit VK. Hair growth: focus on herbal therapeutic agent. *Curr Drug Discov Technol* 2015;12:21-42.
 11. Kang JI, Kim SC, Hyun JH, Kang JH, Park DB, Lee YJ, et al. Promotion effect of *Schisandra nigra* on the growth of hair. *Eur J Dermatol* 2009;19:119-125.
 12. Park HJ, Zhang N, Park DK. Topical application of *Polygonum multiflorum* extract induces hair growth of resting hair follicles through upregulating Shh and β -catenin expression in C57BL/6 mice. *J Ethnopharmacol* 2011;135:369-375.
 13. Rahman MA, Yang H, Lim SS, Huh SO. Apoptotic effects of *Melandrium firmum* root extracts in human SH-SY5Y neuroblastoma cells. *Exp Neurobiol* 2013;22:208-213.
 14. Zheng MS, Hwang NK, Kim DH, Moon TC, Son JK, Chang HW. Chemical constituents of *Melandrium firmum* Rohrbach and their anti-inflammatory activity. *Arch Pharm Res* 2008;31:318-322.
 15. Matsuda H, Sato N, Yamazaki M, Naruto S, Kubo M. Testosterone 5 α -reductase inhibitory active constituents from *Anemarrhenae Rhizoma*. *Biol Pharm Bull* 2001;24:586-587.
 16. Müller-Röver S, Handjiski B, van der Veen C, Eichmüller 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.
 17. Tan JJY, Pan J, Sun L, Zhang J, Wu C, Kang L. Bioactives in Chinese proprietary medicine modulates 5 α -reductase activity and gene expression associated with androgenetic alopecia. *Front Pharmacol* 2017;8:194.
 18. Inui S, Fukuzato Y, Nakajima T, Yoshikawa K, Itami S. Androgen-inducible TGF- β 1 from balding dermal papilla cells inhibits epithelial cell growth: a clue to understand paradoxical effects of androgen on human hair growth. *FASEB J* 2002;16:1967-1969.
 19. Inui S, Fukuzato Y, Nakajima T, Yoshikawa K, Itami S. Identification of androgen-inducible TGF- β 1 derived from dermal papilla cells as a key mediator in androgenetic alopecia. *J Invest Dermatol Symp Proc* 2003;8:69-71.
 20. Kwack MH, Sung YK, Chung EJ, Im SU, Ahn JS, Kim MK, et al. Dihydrotestosterone-inducible dickkopf 1 from balding dermal papilla cells causes apoptosis in follicular keratinocytes. *J Invest Dermatol* 2008;128:262-269.
 21. Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. *Dev Cell* 2002;2:643-653.
 22. Zhao J, Harada N, Kurihara H, Nakagata N, Okajima K. Dietary isoflavone increases insulin-like growth factor-I production, thereby promoting hair growth in mice. *J Nutr Biochem* 2011;22:227-233.
 23. Rajendran RL, Gangadaran P, Bak SS, Oh JM, Kalimuthu S, Lee HW, et al. Extracellular vesicles derived from MSCs activates dermal papilla cell in vitro and promotes hair follicle conversion from telogen to anagen in mice. *Sci Rep* 2017;7:15560.
 24. Werner S, Smola H, Liao X, Longaker MT, Krieg T, Hofschneider PH, et al. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science* 1994;266:819-822.
 25. Jena AK, Vasisht K, Sharma N, Kaur R, Dhingra MS, Karan M. Amelioration of testosterone induced benign prostatic hyperplasia by *Prunus* species. *J Ethnopharmacol* 2016;190:33-45.
 26. Christensen KB, Petersen RK, Kristiansen K, Christensen LP. Identification of bioactive compounds from flowers of black elder (*Sambucus nigra* L.) that activate the human peroxisome proliferator-activated receptor (PPAR) gamma. *Phytother Res* 2010;24 Suppl 2:S129-S132.