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Therapeutic potential of Ishophloroglucin a from *Ishige okamurae* in androgenic alopecia: inhibition of 5α-reductase activity and activation of the Wnt/β-catenin signaling pathway in human dermal papilla cells

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Abstract
Androgenic alopecia (AGA) is a common condition of hair loss, triggered by excessive 5α-dihydrotestosterone (5α-DHT) generated via 5α-reductase activity. This study investigated the anti-alopecia effects of Ishophloroglucin A (IPA), a compound isolated from the brown seaweed *Ishige okamurae*. Molecular docking analysis revealed that IPA exhibits higher binding affinity to 5α-reductase than finasteride. In human dermal papilla cells (HDPCs), IPA inhibited both 5α-reductase activity and androgen receptor (AR) expression, reduced levels of dickkopf-related protein 1 (DKK1), transforming growth factor beta 1 (TGF-β1), and interleukin-6 (IL-6), and activated the Wnt/β-catenin signaling pathway by promoting glycogen synthase kinase-3 beta (GSK3β) phosphorylation and upregulating beta-catenin (β-catenin) expression. Additionally, IPA increased the expression of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), both of which are associated with hair growth promotion. These findings suggest that IPA is a promising therapeutic candidate for treating AGA.

Keywords Androgenic alopecia, Ishige okamurae, Ishophloroglucin a, Human dermal papilla cell, Wnt/β-catenin signaling

Introduction

Androgenic alopecia (AGA) is the most prevalent type of hair loss, affecting men and women worldwide [1, 2]. The condition is primarily attributed to genetic factor and androgen-driven miniaturization hair follicles. Individuals with AGA typically exhibit excessive dihydrotestosterone (DHT) accumulation, heightened 5α-reductase activity, and elevated androgen receptor expression in scalp regions prone to hair loss [3, 4]. This androgen-rich environment disrupts hair follicle homeostasis, resulting in altered hair cycling, prolonged telogen (resting phase), and premature termination of anagen (growth phase),

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which ultimately leads to follicular shrinkage and progressive hair thinning the hallmarks of AGA [5, 6].

Recent many studies highlight the pivotal role of the Wnt/β-catenin signaling pathway in activating and maintenance hair follicle stem cells, thereby regulating hair regeneration and cycling. Dysregulation of this pathway in AGA contributes to follicular aging and ongoing hair loss. Given its crucial role in maintaining follicular integrity, targeting the Wnt/β-catenin axis has emerged as a promising therapeutic strategy to counteract AGA-induced follicular degeneration [7, 8].

Currently, Food and Drug Administration(FDA) approved drugs such as finasteride and minoxidil are widely used to treat AGA. However, these treatments require at least 4 to 6 months of continuous use before visible results appear and must be maintained indefinitely to preserve their effects [9]. In addition, clinical application is hindered by inconsistent efficacy and undesirable side effects such as decreased sexual function, increased skin sensitivity, and scalp dryness, necessitating the exploration of alternative therapeutic agents with enhanced safety and efficacy profiles [10, 11].

As a result, there is an increasing study for safe and effective natural alternatives with hair growth-promoting properties [12]. Marine-derived bioactive compounds have gained attention for their pharmacological potential, particularly in dermatological applications [13, 14].

Among them, polyphenolic phlorotannins from brown seaweeds have demonstrated antioxidant, anti-inflammatory, and hair growth-promoting properties [15, 16]. Ishophloroglucin A (IPA), a phlorotannin isolated from *Ishige okamurae*, has demonstrated various biological activities [17], yet its effects on AGA and hair regrowth remain largely uncharacterized.

Given the critical involvement of the Wnt/β-catenin signaling pathway in hair follicle regeneration, this study aims to investigate the therapeutic potential of IPA in promoting hair growth through the modulation of this pathway in human dermal papilla cells (HDPCs). We found that IPA increased proliferation, migration, and expression of key hair growth markers in hDPCs and activated the Wnt/β-catenin pathway.

We found that IPA increased hDPCs proliferation, migration, and the expression of key hair growth markers, as well as activated the Wnt/β-catenin pathway. These findings offer new insights into the application of IPA as a natural therapeutic agent for AGA, providing an alternative to conventional pharmacological treatments and supporting the broader use of marine-derived functional ingredients in the pharmaceutical and cosmeceutical industries.

Materials and methods

Materials and reagents

3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and antibiotics (penicillin and streptomycin) were purchased from Gibco BRL (Life Technologies, Burlington, ON, Canada). The growth medium were purchased from CEFO$_{gro}$-HDP(CEFO, Seoul, Republic of Korea). Primary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

Molecular docking of 5α-reductase

Molecular docking studies of Ishophloroglucin A(IPA) and finasteride into the type 2 5α-reductase were performed using the flexible docking and the calculated binding energy protocols in Discovery Studio 2024 (Biovia, San Diego, CA, USA). The full protein structure of 5α-reductase (PDB ID: 7BW1) was downloaded from protein data bank (www.rcsb.org). The docking site was defined from the binding pocket of nicotinamide adenine dinucleotide phosphate (NADP)-dihydrofinasteride including entry port (xyz coordinates: 9.81962–1.3815 9.27069, radius 23.5 Å) based on the previous paper [https://doi.org/10.21203/rs.3.rs-40159/v1].

Extraction and isolation of IPA

Ishige okamurae was collected in June 2021 along the eastern coastline of Jeju Island, Korea. The harvested biomass underwent thorough washing with running tap water to eliminate residual salts and sand particles adhered to the surface. The cleaned samples were subsequently subjected to freeze-drying to preserve bioactive constituents. Once dried, the material was finely pulverized using a mechanical grinder and extracted with 70% ethanol to obtain the ethanol-soluble fraction of *I. okamurae* (IOE). Following extraction, IPA was isolated from IOE using a previously established protocol [14]. In brief, fractionation of IOE was conducted via centrifugal partition chromatography, and the collected fractions underwent further purification through a semi-preparative HPLC system equipped with a YMC-Pack ODS-A column (10 mm, 250 mm, 5 μm). The identity of IPA was confirmed by comparing its MS and NMR profiles with the spectral data reported by Ryu et al. (2018). A summary of the reference spectral information is provided in Supplementary Table S1.

Cell culture

Human dermal papilla cells (HDPCs) were obtained from CEFO Co., Ltd. (Seoul, Korea) and maintained in CEFOgro-HDP growth medium, which consists of a basal medium supplemented with essential nutrients and

penicillin/streptomycin. Cells were incubated at 37 °C,5% CO_2 and 95% air.

Measurement of cell proliferation

Cell proliferation was evaluated using an MTT colorimetric assay. HDPCs were seeded in 24-well plates and incubated overnight. The cells were then treated with IPA at 20, 40, and 80 nM, concentrations selected based on preliminary MTT assays showing no cytotoxicity within this range and clear dose-dependent cellular responses. Finasteride (20 nM) was used as a positive control. After 24 h of treatment, MTT solution was added to each well and incubated for 3 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 540 nm using a microplate reader (Synergy HTX Multi-Mode 121 Reader, BioTek Instruments, Winooski, USA).

Measurement of cell migration assay

The cell migration of IPA on cell (HDPCs cell) was assessed using a Culture Insert-2 Well (ibidi GmbH, Munich, Germany). The dish were treated with different concentrations of IPA and Finasteride for 24 h. Proliferation rate was quantified by measuring the remaining uncovered area with the ImageJ software program (Version 1.43; Broken Symmetry Software, Bethesda, MD, USA).

Measurement of 5α-reductase and DHT inhibitory activity

The inhibitory effect of IPA on 5α-reductase activity was evaluated using a purified enzyme–substrate reaction system provided in the Type II 5α-Reductase (SRD5A2) Inhibition Kit (iPhase, IPHA-SRD5A2-KIT, Guangzhou, China). In this assay, recombinant human SRD5A2 catalyzes the NADPH-dependent reduction of testosterone to dihydrotestosterone (DHT), and inhibition was quantified by calculating IC_{50} values according to the manufacturer's procedure. To determine the effect of IPA on androgen-induced DHT production in cells, human dermal papilla cells (HDPCs) were stimulated with testosterone and treated with IPA or finasteride. Intracellular DHT levels were measured using a Human Dihydrotestosterone (DHT) ELISA Kit (Abebio, Wuhan, China) following the manufacturer's instructions.

Measurement of TGF-β1 and IL-6 inhibitory activity

The TGF-β1 and IL-6 inhibitory activity of IPA in HDPCs cells was assessed using the human TGF-β1 and IL-6 ELISA kit (R&D Systems, Minneapolis, MN, USA), following the recommended procedures.

Measurement of VEGF and FGF activity

The VEGF and FGF activity of IPA in HDPCs cells was assessed using the human VEGF and FGF ELISA kit (R&D Systems, Minneapolis, MN, USA), following the recommended procedures.

Western blot analysis

The molecular mechanisms underlying the effects of IPA on hair follicle regeneration were assessed by evaluating protein expression levels in HDPCs. Following cell lysis with RIPA buffer, lysates were obtained by centrifugation at 13,000 rpm for 15 min at 4 °C. Equal protein concentrations of the samples were loaded onto a 15% SDS-PAGE gel for electrophoretic separation and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% nonfat dry milk in TBS-T (TBS containing 0.05% tween, pH 7.4) for 1.5 h at room temperature and subsequently incubated with primary antibodies for overnight at 4℃. After washing with TBS-T, the membranes were treated with secondary antibodies for 2 h at RT. Protein bands were detected using a chemiluminescence imaging system (FUSION SOLO, Vilber Lourmat, Paris, France) and quantified with ImageJ software.

Statistical analysis

All the experiments were conducted in triplicate. The data are expressed as mean ± standard error (SD) and analyzed by one-way analysis of variance (ANOVA). Analysis of the results was performed using the SPSS statistical program (Version 28, IBM, Armonk, NY, USA). Significant differences ($p < 0.05$) between the groups were identified using the Tukey post hoc test.

Results

Molecular docking analysis

We assessed the inhibitory capacity of IPA against human type II 5α-reductase (PDB ID: 7BW1) in comparison with finasteride. As summarized in Table 1 and illustrated in Fig. 1, the calculated binding free energy of the 5α-reductase IPA complex was markedly more favorable than that of the 5α-reductase–finasteride complex. Within the catalytic pocket, IPA engaged in a diverse network of interactions, including three salt-bridge contacts with Lys29, Arg105, and Lys237; six conventional hydrogen bonds formed with Glu36, Ser6, Lys237, Val27, Lys29, and Arg105; two cation–π interactions with Arg105 and Arg114; and six π–alkyl hydrophobic contacts involving Phe227, Phe234, Tyr178, Tyr183, Leu23, and Leu111. IPA was additionally stabilized by extensive van der Waals contacts with Met236, Gly32, Gly115, Thr4, Gln56, Trp35, and Tyr98. In contrast, finasteride displayed a much simpler interaction profile, forming only three conventional hydrogen bonds with Glu197, Asn160, and Asn193, along with four π–alkyl hydrophobic contacts at Phe118, Phe223, Phe194, and Leu224, and several van der Waals contacts. A previous study presented the binding

Table 1 Comparative binding energy of finasteride and IPA for the active site of 5α-reductase

	IPA	Finasteride
Binding energy(kcal/mol)	−1354.85	−81.385
Hydrogen bond	GLU36, LYS237, SER6, LYS29, VAL27, PHE234, ARG105	GLU197, ASN160, ASN193
Salt bridge	ARG105, LYS237, LYS29	Not observed
Conventional H-bond	GLU36, SER6, LYS237, VAL27, LYS29, ARG105	GLU197, ASN160, ASN193
Cation–π interaction	ARG114, ARG105	Not observed
π–π T-shaped stacking	PHE223, LEU23	Not observed
π–Alkyl hydrophobic contact	PHE227,PHE234, TYR178, TYR183, LEU23, LEU111	PHE118,PHE223, PHE194, LEU224
van der Waals contacts	MET236, GLY32, GLY115, THR4, GLN56, TRP35, TYR98	GLY115, GLN56, GLN33, SER31, GLU57, ARG94, ASP164

site and entry port of NADP-dihydrofinasteride, a substrate of 5α-reductase that includes Leu23, Tyr33, Trp53, Leu111, and Phe223 [18]. IPA is bonded to the entire NADP-dihydrofinasteride binding pocket, having entered through the entry port and covered the surface of the NADP binding site. These results demonstrate that IPA exhibits a substantially broader and stronger interaction repertoire within the 5α-reductase active site, suggesting superior inhibitory potential compared with finasteride.

Cell proliferation and migration effect of IPA in HDPCs cells

To establish non-cytotoxic dosing, HDPCs cells were exposed to IPA (20, 40, and 80 nM) and finasteride (20 nM) for 24 h and viability was assessed by MTT assay (Fig. 2A). IPA treatment at concentrations of 20, 40, and 80 nM did not affect HDPCs viability, maintaining over 95% survival compared to controls. We next performed a scratch assay to evaluate IPA and finasteride effect on hDPCs cells migration. Confluent monolayers were scratched, treated with IPA and finasteride and imaged at 24 h (Fig. 2B). Quantitative analysis of proliferation rate (Fig. 2C) showed a concentration-dependent increase in migration: each IPA dose significantly accelerated gap closure compared to untreated (control) group (***$p < 0.001$).

5α-reductase inhibitory activity and effects of IPA on DHT production in testosterone-stimulated hDPCs

5α-Reductase catalyzes the NADPH-dependent conversion of testosterone to dihydrotestosterone (DHT), a key androgen implicated in the development of androgenic alopecia [3, 4]. Based on the strong binding interaction predicted in our molecular docking analysis, we next examined whether IPA could inhibit 5α-reductase activity in testosterone-stimulated HDPCs. As presented in Table 2, IPA showed an IC_{50} value of 304.14 μM, which was lower than that of finasteride (506.10 μM), indicating a comparatively stronger inhibitory tendency toward type II 5α-reductase.

We then evaluated the downstream consequence of this enzymatic inhibition by measuring intracellular DHT levels. As shown in Fig. 3, IPA significantly reduced DHT production in a dose-dependent manner. DHT levels were markedly lower compared with both the untreated control group (###$p < 0.001$) and the testosterone-only group (***$p < 0.001$). These findings confirm that IPA suppresses testosterone-to-DHT conversion in HDPCs, consistent with its higher inhibitory potency toward 5α-reductase.

Effects of IPA on growth factor in testosterone-stimulated HDPCs cells

Insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) are key paracrine mediators secreted by dermal papilla cells to drive proliferation and perifollicular angiogenesis [19]. As depicted in Fig. 4A and B, exposure to testosterone (20 nM) markedly reduced secretion of both IGF-1 and VEGF in hDPCs cells relative to untreated controls (#$p < 0.05$). Subsequent treatment with IPA (20–80 nM) restored IGF-1 and VEGF levels in a dose-dependent manner, with IPA (80 nM) significantly increasing levels compared to the testosterone-only group. These results indicate that IPA effectively reverses the androgen-induced downregulation of IGF-1 and VEGF, thereby restoring a pro-anabolic milieu favourable for hair follicle growth.

Cytokine productions inhibitory activity of IPA in testosterone-stimulated HDPCs cells

Cytokines such as transforming growth factor-β1 (TGF-β1) and interleukin-6 (IL-6) are upregulated by the androgen receptor–DHT complex during the onset of androgenic alopecia. These mediators drive premature entry of hair follicles into the telogen phase and promote keratinocyte apoptosis, thereby impairing follicular growth [20–22]. As shown in Fig. 5A, testosterone stimulation caused a marked elevation of TGF-β1 in hDPCs cells relative to untreated controls. Subsequent treatment with IPA (20–80 nM) resulted in a concentration-dependent suppression of TGF-β1, with the IPA (20–80 nM) dose eliciting a significantly greater reduction than finasteride. In parallel, IL-6 overproduction induced by testosterone was effectively attenuated by IPA across the tested concentrations (Fig. 5B). These findings suggest that IPA effectively counteracts androgen-induced inflammatory responses.

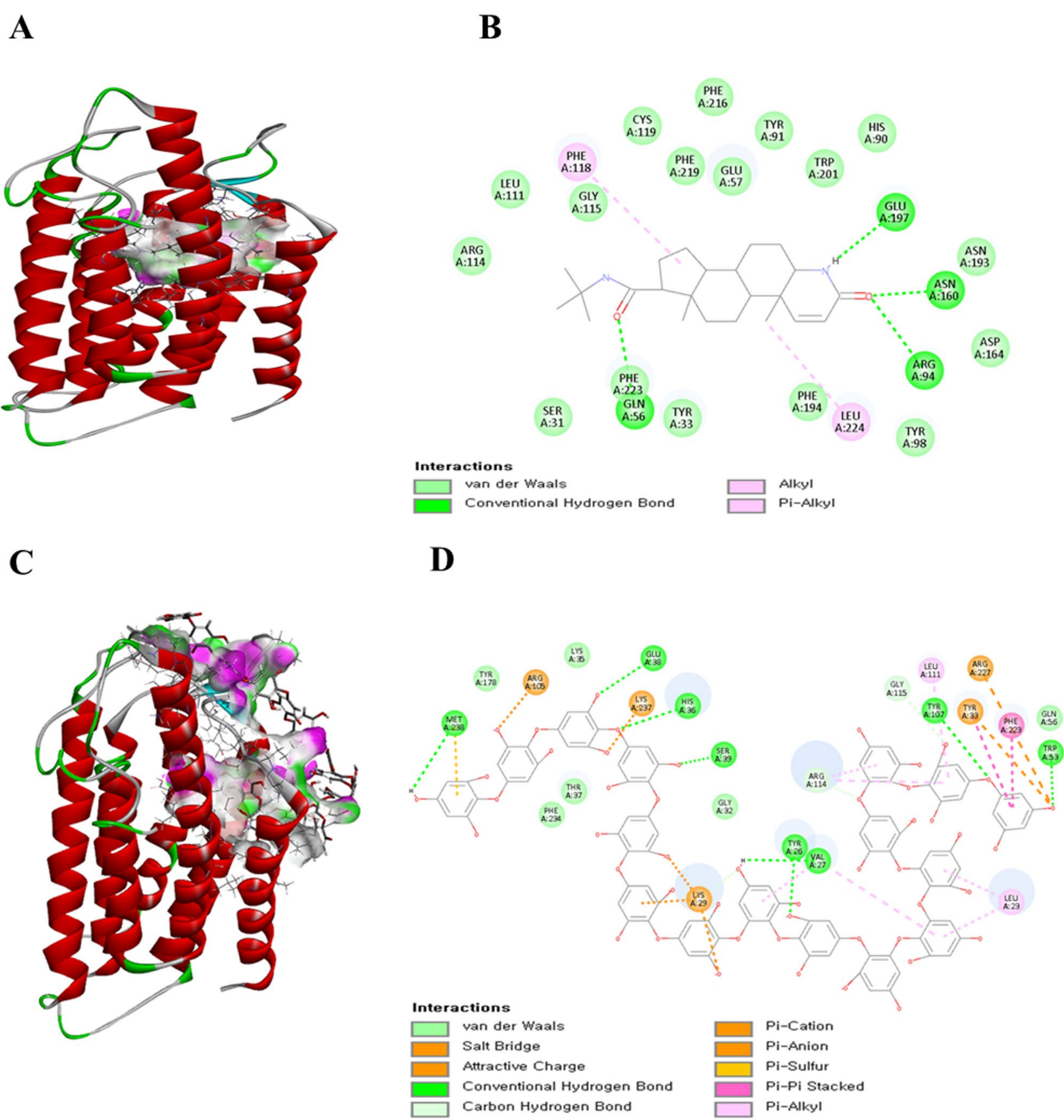

Fig. 1 Computational prediction of IPA, finasteride and type 2 5α-reductase by molecular docking analysis. 3D structure of type 2 5α-reductase-finasteride complex (**A**), 2D view of the type 2 5α-reductase-finasteride complex (**B**), 3D structure of type 2 5α-reductase-IPA complex (**C**), and 2D view of the type 2 5α-reductase-IPA complex (**D**) are shown

Effects of IPA on androgen receptor (AR) signaling pathway in testosterone-stimulated HDPCs cells

In androgenic alopecia, testosterone is converted by 5α-reductase into DHT, which binds AR and drives DKK1 expression; DKK1 then antagonizes Wnt/β-catenin signaling, precipitating follicular miniaturization [23, 24]. To investigate whether Ishophloroglucin A (IPA) could counteract this cascade, we performed western blotting for AR and DKK1 in testosterone-stimulated hDPCs cells. As expected, testosterone significantly upregulated both AR and DKK1 compared to vehicle controls. Co-treatment with IPA (20–80 nM) resulted in a concentration-dependent suppression of these proteins, with IPA achieving a greater reduction in AR pathway activation (Fig. 6). These results indicate that IPA effectively attenuates androgen-induced AR activation and subsequent DKK1 overexpression, thereby supporting

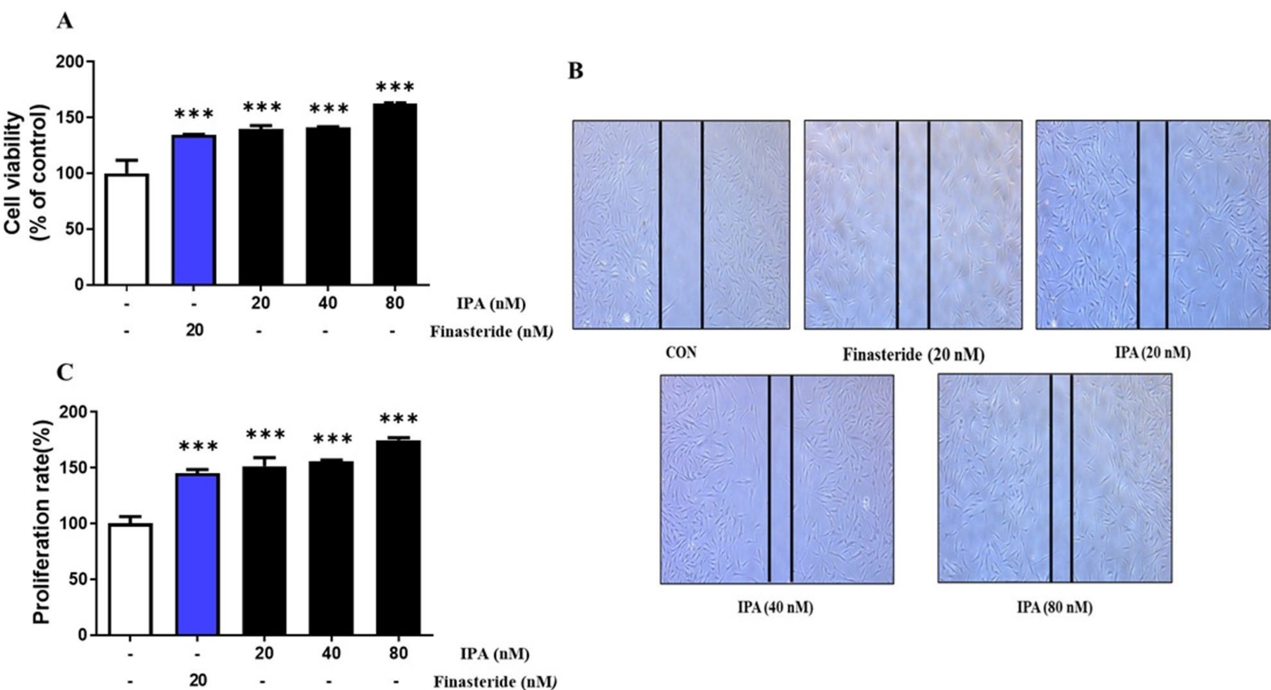

Fig. 2 Cell proliferation effect of IPA and finasteride in HDPCs cells. Cell viability was determined by MTT assay (**A**), photomicrographs of culture plates were taken directly using a phase-contrast microscope (20× magnification) (**B**) closure rates of both cell lines (**C**). Values are expressed as mean ± SD of triplicate experiments. ***$p < 0.001$ indicate significant differences compared with the untreated (control) group

Table 2 IC_{50} values for 5α-reductase Inhibition by finasteride and IPA

	IPA	Finasteride
IC_{50} values (μM)	304.14	506.10

the maintenance of Wnt/β-catenin signaling and hair follicle health.

Effects of IPA on Wnt/β-catenin signaling pathway in testosterone-stimulated HDPCs cells

Hair follicle homeostasis relies on coordinated activation of the Wnt/β-catenin pathways, where AKT

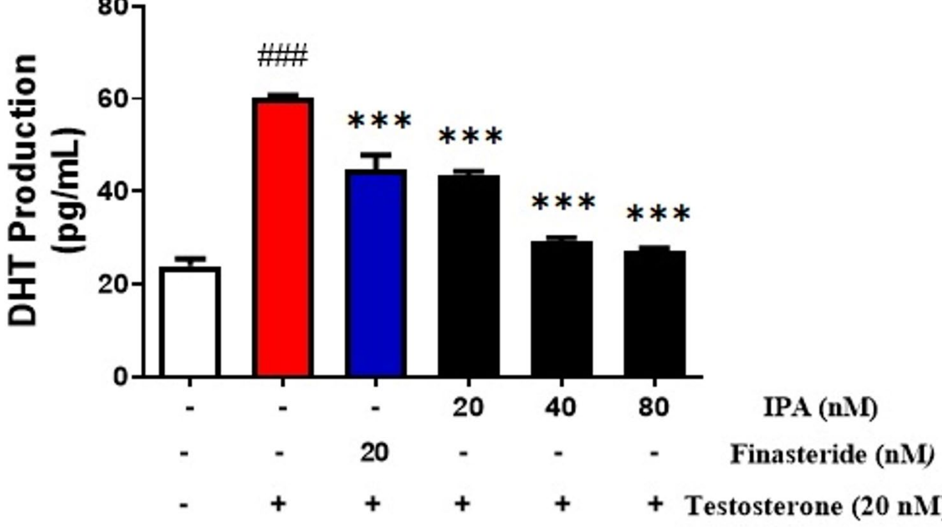

Fig. 3 DHT Production inhibitory activity of IPA in testosterone-stimulated HDPCs cells. The 5α-reductase inhibitory activity was determined using an ELISA kit. Values are expressed as mean ± SD of triplicate experiments. ###$p < 0.001$ indicate significant differences compared to the untreated (control) group and ***$p < 0.001$ compared to the testosterone_treated group

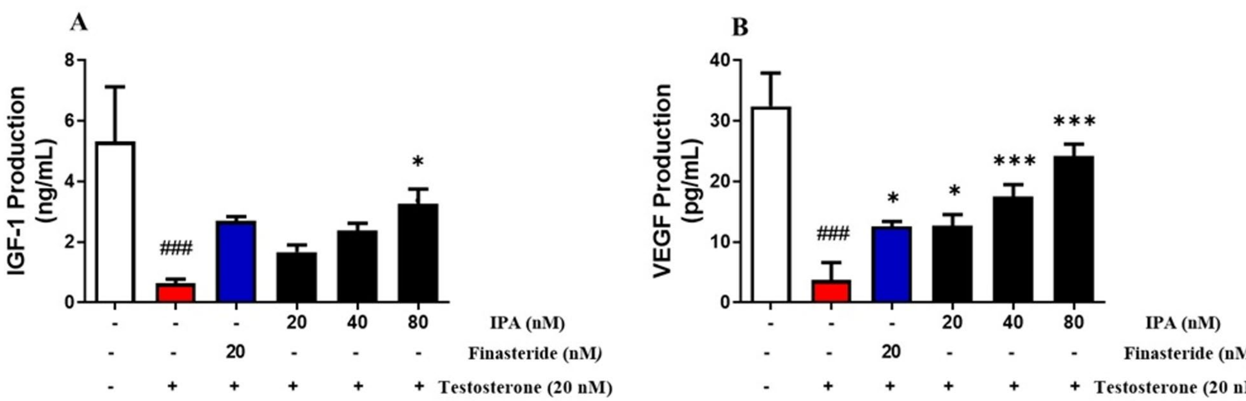

Fig. 4 Growth factor production activity of IPA in testosterone-stimulated HDPCs cells. Growth factor (**A**) IGF-1 and (**B**) VEGF production activity was determined using an ELISA kit. Values are expressed as mean ± SD of triplicate experiments. ###$p < 0.001$ indicate significant differences compared to the untreated (control) group and *$p < 0.05$, ***$p < 0.001$ compared to the testosterone_treated group

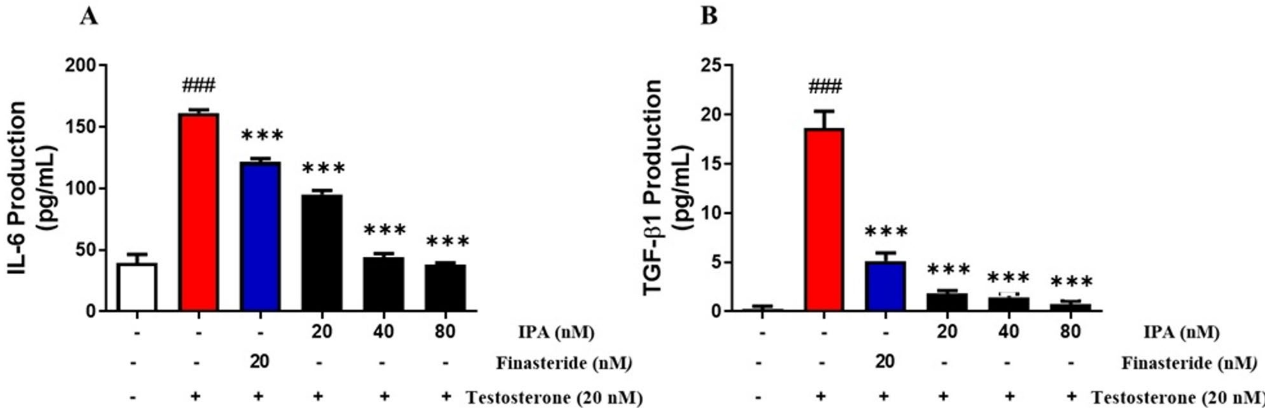

Fig. 5 Cytokine production inhibitory activity of IPA in testosterone-stimulated HDPCs cells. Cytokine production IL-6 (**A**) and TGF-β1 (**B**) inhibitory activity was determined using an ELISA kit. Values are expressed as mean ± SD of triplicate experiments. ###$p < 0.001$ indicate significant differences compared to the untreated (control) group and ***$p < 0.001$ compared to the testosterone_treated group

phosphorylation enhances cell survival and GSK3β inhibition permits β-catenin stabilization to drive proliferation [25, 26]. To assess the ability of IPA to reactivate these axes under androgenic stress, HDPCs cells were treated with testosterone (20 nM) followed by IPA (20, 40, 80 nM) or finasteride (20 nM) and key signaling nodes were assessed by western blot (Fig. 7). Testosterone significantly suppressed p-AKT, p-GSK3β, β-catenin and PCNA compared to vehicle controls. IPA treatment restored all four markers in a clear dose-dependent manner, with the highest IPA concentration providing a much greater recovery than finasteride at the same dose (*$p < 0.05$). These results demonstrate that IPA more effectively reactivates the Wnt/β-catenin pathway, which is essential for hair follicle regeneration.

Discussion

Androgenetic alopecia (AGA) is primarily attributed to androgen-related hormonal imbalances, which promote gradual, non-scarring miniaturization of hair follicles and shorten the anagen phase in genetically predisposed men and women [1]. Central to the pathogenesis of AGA is the excessive accumulation of dihydrotestosterone (DHT) due to upregulated type II 5α-reductase activity [3, 4]. As a result, pharmacological inhibition of 5α-reductase has become a key strategy in AGA management. Finasteride, a competitive type II 5α-reductase inhibitor, is approved as a first-line treatment to slow follicular miniaturization and maintain hair density [9]. however, its use has been linked to a range of adverse effects, including sexual, neurological, psychiatric, endocrine, metabolic, and ophthalmological complications, as well as an increased risk of high-grade prostate cancer [10, 11]. Therefore, the increased use of drugs for the increasing prevalence of hair loss in recent years has resulted in significant physical and psychological burdens, leading to research into innovative treatment strategies [27, 28].

Consequently, there is growing interest in natural bioactive compounds such as polysaccharides, proteins, lipids, and polyphenols as safer alternatives for managing

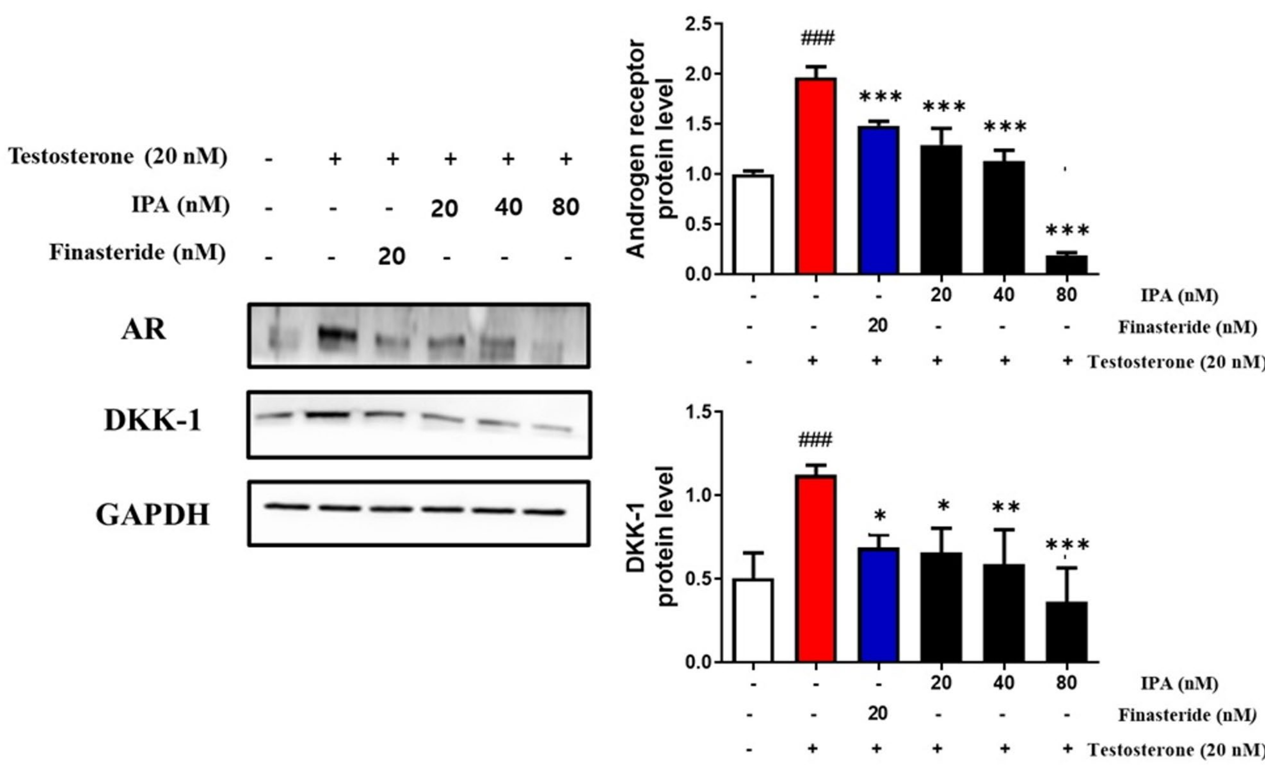

Fig. 6 The effects of IPA on the expression levels of regulatory proteins in androgen receptor (AR) signaling pathway. The expression levels of AR (Androgen receptor) and dickkopf-related protein 1 (DKK1) were measured by western blot analysis. Values are expressed as mean ± SD of triplicate experiments. ###$p < 0.001$ indicate significant differences compared to the untreated (control) group and *$p < 0.05$, ***$p < 0.001$ compared to the testosterone_treated group

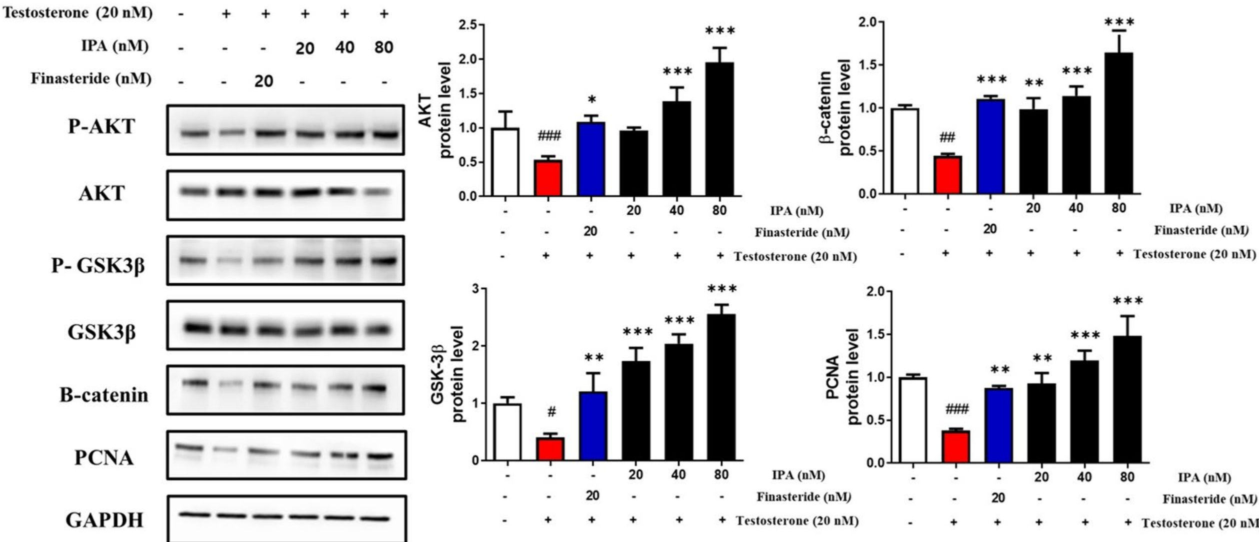

Fig. 7 The effects of IPA on the expression levels of regulatory proteins in Wnt/β-catenin signaling pathway. The expression levels of the proteins were measured by western blot analysis. Values are expressed as mean ± SD of triplicate experiments. ###$p < 0.001$, ##$p < 0.01$ indicate significant differences compared to the untreated (control) group and ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ compared to the testosterone_treated group

hair loss [29, 30]. Notably, marine-derived molecules have shown potential to inhibit 5α-reductase, stimulate dermal papilla cell proliferation, and promote anagen entry by modulating the Wnt/β-catenin pathway [31, 32]. Ishophloroglucin A (IPA), a phlorotannin isolated from the brown alga *I. okamurae*, has demonstrated diverse biological activities and an excellent safety profile in both in vitro and in vivo studies [17]. However, its efficacy in

counteracting androgen-induced hair follicle disruption remains largely unexplored. The present study first demonstrates that IPA exerts potent anti-androgenic alopecia effects in HDPCs cell by targeting multiple pathological mechanisms underlying AGA.

Molecular docking has become an essential computational approach in early-phase drug discovery, offering valuable insights into how small molecules interact with their target proteins at the molecular level. This technique not only simulates the spatial fit of ligands within enzymatic active sites but also estimates the strength and specificity of their interactions, providing a rational basis for predicting pharmacological activity [33, 34]. In the present study, molecular docking simulations were conducted to examine the binding characteristics of IPA toward type II 5α-reductase, a key enzyme implicated in the pathophysiology of AGA. IPA formed an extensive interaction network within the catalytic pocket, including three salt bridges, six hydrogen bonds, two cation–π interactions, two T-shaped π–π stackings, six π–alkyl interactions, and numerous van der Waals contacts. In contrast, finasteride exhibited clearly fewer interactions, forming only one hydrogen bond and two alkyl contacts, along with a markedly higher docking energy score.

These in silico findings were further substantiated by a purified enzyme-based assay: IPA demonstrated a lower IC_{50} value (304.14 μM) than finasteride (506.10 μM), indicating superior inhibitory capacity toward type II 5α-reductase. Consistently, IPA suppressed testosterone-to-DHT conversion in a dose-dependent manner, confirming that the strong binding affinity predicted in the docking simulation translates into functional inhibition of the enzyme. Since type II 5α-reductase catalyzes the NADPH-dependent reduction of testosterone to DHT, blockade of this enzymatic step directly accounts for the reduced DHT levels observed in the assay [3, 4]. Together, these findings demonstrate that IPA acts as an effective enzymatic inhibitor and provides a biochemical basis for its downstream biological activities observed in HDPCs. However, the present dataset does not clarify whether IPA inhibits 5α-reductase through a competitive or non-competitive mechanism. Because IC_{50} values and endpoint DHT measurements cannot distinguish inhibition types, detailed kinetic analyses employing variable substrate concentrations will be required to define its precise mode of inhibition. Although molecular docking strongly suggested that IPA interacts directly with the catalytic pocket of 5α-reductase, the present study did not include a direct target-engagement assay, such as DARTS, pull-down, or finasteride-competition analysis. The functional evidence obtained from the purified enzyme assay and the suppression of testosterone-to-DHT conversion supports that IPA acts on 5α-reductase; however, biochemical validation of physical binding is still required. Future studies incorporating DARTS or competition assays with finasteride will be essential to confirm that IPA occupies the same binding pocket and to elucidate its precise interaction mode with SRD5A2.

During the anagen phase, vascular endothelial growth factor (VEGF)-mediated angiogenesis plays a critical role in delivering oxygen and nutrients to actively growing hair follicles, thereby influencing follicle size and hair shaft diameter [35]. Insulin-like growth factor 1 (IGF-1) has also been identified as a key regulator of hair follicle development, sustaining anagen progression and modulating the transition to catagen. Dysregulation of IGF-1 signaling has been implicated in the pathogenesis of AGA [36, 37]. Mechanistically, IGF-1 promotes the proliferation of dermal papilla cells and inhibits apoptosis, thereby contributing to follicular maintenance and survival [38]. In the present study, IPA treatment significantly restored IGF-1 and VEGF levels in testosterone-stimulated human dermal papilla cells (HDPCs), compared with testosterone treatment alone. This restoration of key trophic factors suggests that IPA may help to reestablish a follicular microenvironment favorable for growth and regeneration under androgenic stress. Additionally, IPA effectively suppressed testosterone-induced upregulation of pro-inflammatory cytokines, including transforming growth factor-β1 (TGF-β1) and interleukin-6 (IL-6), both of which are known to trigger premature follicular regression and apoptosis. These findings support the potential of IPA to attenuate the inflammatory microenvironment associated with AGA progression and maintain hair follicle integrity.

AGA is closely associated with alterations in the Wnt/β-catenin signaling pathway, which is essential for the regulation of hair follicle morphogenesis and cycling. This pathway governs the proliferation and differentiation of follicular cells [39]. However, this signaling axis can be disrupted by androgenic stimuli particularly by dihydrotestosterone (DHT) which enhances the activity of glycogen synthase kinase-3 beta (GSK3β), a negative modulator of the Wnt pathway. Activated GSK3β sequentially phosphorylates β-catenin at specific residues (Thr41, Ser37, and Ser33), marking it for ubiquitination and subsequent degradation via the proteasome system [40]. In contrast, phosphorylation at Ser9 inhibits GSK3β activity, thereby preserving β-catenin stability and sustaining Wnt signaling [41].

To explore the modulatory potential of IPA on androgen-disrupted pathways, we conducted an in vitro evaluation using human dermal papilla cells (HDPCs). Testosterone exposure heightened the activity of GSK3β, which in turn led to a decrease in β-catenin levels a hallmark of impaired Wnt/β-catenin signaling. Remarkably, IPA administration reversed these alterations in a dose-dependent fashion, evident through reduced

phosphorylation of GSK3β at activating residues and restored β-catenin expression. These findings indicate that IPA can mitigate androgen-induced suppression of Wnt signaling, supporting its role in maintaining hair follicle integrity. Notably, this regulatory pattern is consistent with other marine-derived phlorotannins, such as diphlorethohydroxycarmalol (DPHC), which have previously shown similar bioactivity in HDPCs [15]. This suggests a broader therapeutic potential of marine polyphenols in targeting multiple hair loss mechanisms with improved safety profiles.

In conclusion, IPA isolated from *I. okamurae* demonstrated a multi-pronged mechanism of action against androgenetic alopecia. It not only inhibited type II 5α-reductase activity but also reactivated suppressed growth factors such as VEGF and IGF-1, modulated pro-inflammatory cytokines, and restored Wnt/β-catenin signaling in HDPCs cell. These combined effects suggest that IPA holds strong promise as a naturally derived candidate for treating AGA. Future preclinical and clinical studies are warranted to validate these effects and explore its translation into therapeutic application.

Abbreviations

AGA	Androgenic alopecia
AR	Androgen receptor
AKT	Protein kinase B
β-catenin	Beta-catenin
DHT	Dihydrotestosterone
DKK1	Dickkopf-related protein 1
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FGF	Fibroblast growth factor
GSK3β	Glycogen synthase kinase-3 beta
hDPCs	Human dermal papilla cells
HPLC	High-performance liquid chromatography
IGF-1	Insulin-like growth factor-1
IL-6	Interleukin-6
IPA	Ishophloroglucin A
IOE	Ishige okamurae ethanol extract
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PCNA	Proliferating cell nuclear antigen
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TBS-T	Tris-buffered saline with Tween 20
TGF-β1	Transforming growth factor-beta 1
VEGF	Vascular endothelial growth factor
Wnt	Wingless-type MMTV integration site family

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Author contributions
Wook-Chul Kim: Writing – original draft, Methodology, Investigation, Formal analysis. Hyun-Soo Kim: Investigation, Formal analysis Nalae Kang: Formal analysis, Methodology· Soo-Jin Heo: Investigation, Formal analysis, Conceptualization. Writing – review & editing, Seung-Hong Lee: Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

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Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that there is no conflict of interest.

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References

1. Sasaki GH (2019) Review of human hair follicle biology: dynamics of niches and stem cell regulation for possible therapeutic hair stimulation for plastic surgeons. Aesthet Plast Surg 43:253–266. https://doi.org/10.1007/s00266-018-1248-1
2. Neuhaus K, Schiestl C, Adelsberger R, Weibel L, Meuli M, Böttcher-Haberzeth S (2019) Bold to do – bald to be? Outcomes decades after harvesting the scalp in burned children. Burns 45:543–553. https://doi.org/10.1016/j.burns.2018.09.023
3. Lolli F, Pallotti F, Rossi A, Fortuna MC, Caro G, Lenzi A, Sansone A, Lombardo F (2017) Androgenetic alopecia: a review. Endocrine 57:9–17. https://doi.org/10.1007/s12020-017-1280-y
4. Rossi A, Anzalone A, Fortuna MC, Caro G, Garelli V, Pranteda G, Carlesimo M (2016) Multi-therapies in androgenetic alopecia: review and clinical experiences. Dermatol Ther 29:424–432. https://doi.org/10.1111/dth.12390
5. Bienenfeld A, Azarchi S, Lo Sicco K, Marchbein S, Shapiro J, Nagler AR (2019) Androgens in women: androgen-mediated skin disease and patient evaluation. J Am Acad Dermatol 80:1497–1506. https://doi.org/10.1016/j.jaad.2018.08.062
6. Sinclair R, Torkamani N, Jones L (2015) Androgenetic alopecia: new insights into the pathogenesis and mechanism of hair loss. F1000Res 4(F1000 Faculty Rev):585. https://doi.org/10.12688/f1000research.6401.1
7. Wang C, Zang K, Tang Z, Yang T, Ye X, Dang Y (2023) Hordenine activated dermal papilla cells and promoted hair regrowth by activating Wnt signaling pathway. Nutrients 15:694. https://doi.org/10.3390/nu15030694
8. Rishikaysh P, Dev K, Diaz D, Qureshi WMS, Filip S, Mokry J (2014) Signaling involved in hair follicle morphogenesis and development. Int J Mol Sci 15:1647–1670. https://doi.org/10.3390/ijms15011647
9. Adil A, Godwin M (2017) The effectiveness of treatments for androgenetic alopecia: a systematic review and meta-analysis. J Am Acad Dermatol 77:136-141.e5. https://doi.org/10.1016/j.jaad.2017.02.054
10. Nestor MS, Ablon G, Gade A, Han H, Fischer DL (2021) Treatment options for androgenetic alopecia: efficacy, side effects, compliance, financial considerations, and ethics. J Cosmet Dermatol 20:3759–3781. https://doi.org/10.1111/jocd.14537
11. Arif T, Dorjay K, Adil M, Sami M (2017) Dutasteride in androgenetic alopecia: an update. Curr Clin Pharmacol 12:31–35. https://doi.org/10.2174/1574884712666170310111125
12. Bacqueville D, Lévêque M, Mas C, Haure MJ, Noustens A, Mengeaud V et al (2024) New plant extracts exert complementary anti-hair loss properties in human in vitro and ex vivo models. J Cosmet Dermatol 23(5):1–11. https://doi.org/10.1111/jocd.16616
13. Kim WC, Kang H, Lee SH (2025) Liquid chromatography quadrupole time-of-flight tandem mass spectrometry characterization of ethyl acetate fraction from *sargassum pallidum* and its anti-melanogenesis effect in B16F10 melanoma cells and zebrafish model. Int J Mol Sci 26:1522. https://doi.org/10.3390/ijms26041522
14. Ryu B, Jiang Y, Kim HS, Hyun JM, Lim SB, Li Y, Jeon YJ (2018) Ishophloroglucin A, a novel phlorotannin for standardizing the anti-α-glucosidase activity of *Ishige okamurae*. Mar Drugs 16:436. https://doi.org/10.3390/md16110436
15. Im ST, Mun H, Kang N, Heo SJ, Lee SH (2025) Anti-androgenetic effect of diphlorethohydroxycarmalol on testosterone-induced hair loss by inhibiting

5α-reductase and promoting Wnt/β-catenin signaling pathway in human dermal papilla cells. Toxicol In Vitro 104:106017. https://doi.org/10.1016/j.tiv.2025.106017
16. Gisbert M, Franco D, Sineiro J, Moreira R (2023) Antioxidant and antidiabetic properties of phlorotannins from *Ascophyllum nodosum* seaweed extracts. Molecules 28:4937. https://doi.org/10.3390/molecules28134937
17. Yang HW, Oh S, Chung DM et al (2022) Ishophloroglucin A, isolated from *Ishige okamurae*, alleviates dexamethasone-induced muscle atrophy through muscle protein metabolism in vivo. Mar Drugs 20:280. https://doi.org/10.3390/md20050280
18. Xiao Q, Wang L, Supekar S et al (2020) Structure of human steroid 5α-reductase 2 with the anti-androgen drug finasteride. Nat Commun 11:5430. https://doi.org/10.1038/s41467-020-19249-z
19. Natarelli N, Gahoonia N, Sivamani RK (2023) Integrative and mechanistic approach to the hair growth cycle and hair loss. J Clin Med 12:893. https://doi.org/10.3390/jcm12030893
20. Hoffmann R, Eicheler W, Huth A et al (1996) Cytokines and growth factors influence hair growth in vitro. Possible implications for the pathogenesis and treatment of alopecia areata. Arch Dermatol Res 288:153–156. https://doi.org/10.1007/BF02505825
21. Kwon YE, Choi SE, Park KH (2022) Regulation of cytokines and dihydrotestosterone production in human hair follicle papilla cells by supercritical extraction-residues extract of ulmus Davidiana. Molecules 27:1419. https://doi.org/10.3390/molecules27041419
22. Kwack MH et al (2012) Dihydrotestosterone-inducible IL-6 inhibits elongation of human hair shafts by suppressing matrix cell proliferation and promotes regression of hair follicles in mice. J Invest Dermatol 132:43–49. https://doi.org/10.1038/jid.2011.274
23. Mao Y, Liu P, Wei J et al (2023) Cell therapy for androgenetic alopecia: elixir or trick? Stem Cell Rev Rep 19:1785–1799. https://doi.org/10.1007/s12015-023-10532-2
24. Shin DW (2022) The molecular mechanism of natural products activating Wnt/β-catenin signaling pathway for improving hair loss. Life 12:1856. https://doi.org/10.3390/life12111856
25. Kishimoto J, Burgeson RE, Morgan BA (2000) Wnt signaling maintains the hair-inducing activity of the dermal papilla. Genes Dev 14:1181–1185. https://doi.org/10.1101/gad.14.10.1181
26. Soma T, Fujiwara S, Shirakata Y, Hashimoto K, Kishimoto J (2012) Hair-inducing ability of human dermal papilla cells cultured under Wnt/β-catenin signalling activation. Exp Dermatol 21:307–309. https://doi.org/10.1111/j.1600-0625.2012.01458.x
27. Wolff H, Fischer TW, Blume-Peytavi U (2016) The diagnosis and treatment of hair and scalp diseases. Dtsch Arztebl Int 113:377–386. https://doi.org/10.3238/arztebl.2016.0377
28. Maloh J, Engel T, Natarelli N, Nong Y, Zufall A, Sivamani RK (2023) Systematic review of psychological interventions for quality of life, mental health, and hair growth in alopecia areata and scarring alopecia. J Clin Med 12:964. https://doi.org/10.3390/jcm12030964
29. Huang CY et al (2022) Hair growth-promoting effects of Sargassum glaucescens oligosaccharides extracts. J Taiwan Inst Chem Eng 134:104307. https://doi.org/10.1016/j.jtice.2022.104307
30. Shen YL, Li XQ, Pan RR, Yue W, Zhang LJ, Zhang H (2018) Medicinal plants for the treatment of hair loss and the suggested mechanisms. Curr Pharm Des 24:3090–3100. https://doi.org/10.2174/1381612824666180723094200
31. Radhika P, Cabeza M, Bratoeff E, García G (2004) 5Alpha-reductase inhibition activity of steroids isolated from marine soft corals. Steroids 69:439–444. https://doi.org/10.1016/j.steroids.2004.04.007
32. Yang F, Jin S, Tang Y (2019) Marine collagen peptides promote cell proliferation of NIH-3T3 fibroblasts via NF-κB signaling pathway. Molecules 24:4201. https://doi.org/10.3390/molecules24224201
33. Sahu D, Rathor LS, Dwivedi SD, Shah K, Chauhan NS, Singh MR, Singh D (2024) A review on molecular docking as an interpretative tool for molecular targets in disease management. Assay Drug Dev Technol 22:40–50. https://doi.org/10.1089/adt.2023.0120
34. Şenol H, Ghaffari-Moghaddam M, Toraman GÖA, Güller U (2024) Novel chalcone derivatives of ursolic acid as acetylcholinesterase inhibitors: synthesis, characterization, biological activity, ADME prediction, molecular docking and molecular dynamics studies. J Mol Struct 1295:136804. https://doi.org/10.1016/j.molstruc.2023.136804
35. Yum S, Jeong S, Kim D et al (2017) Minoxidil induction of VEGF is mediated by inhibition of HIF-prolyl hydroxylase. Int J Mol Sci 19:53. https://doi.org/10.3390/ijms19010053
36. Li J, Yang Z, Li Z et al (2014) Exogenous IGF-1 promotes hair growth by stimulating cell proliferation and down regulating TGF-β1 in C57BL/6 mice in vivo. Growth Horm IGF Res 24:89–94. https://doi.org/10.1016/j.ghir.2014.03.004
37. Castela M, Linay F, Roy E et al (2017) IGF1R signalling acts on the anagen-to-catagen transition in the hair cycle. Exp Dermatol 26:785–791. https://doi.org/10.1111/exd.13287
38. Trüeb RM (2018) Further clinical evidence for the effect of IGF-1 on hair growth and alopecia. Skin Appendage Disord 4:90–95. https://doi.org/10.1159/000479333
39. Leiros GJ, Attorresi AI, Balana ME (2012) Hair follicle stem cell differentiation is inhibited through cross-talk between Wnt/β-catenin and androgen signalling in dermal papilla cells from patients with androgenetic alopecia. Br J Dermatol 166:1035–1042. https://doi.org/10.1111/j.1365-2133.2012.10856.x
40. Hagen T, Vidal-Puig A (2002) Characterisation of the phosphorylation of β-catenin at the GSK-3 priming site Ser45. Biochem Biophys Res Commun 294:324–328. https://doi.org/10.1016/S0006-291X(02)00485-0
41. Huang J, Guo X, Li W et al (2017) Activation of Wnt/β-catenin signalling via GSK3 inhibitors direct differentiation of human adipose stem cells into functional hepatocytes. Sci Rep 7:40716. https://doi.org/10.1038/srep40716

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