



Article

A Platform for Determining Medicinal Plants with Targeted 17β -Hydroxysteroid Dehydrogenase Modulation for Possible Hair Loss Prevention

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Abstract: Although hair loss plays a vital physiological function in present society, their impact on shaping self-esteem is undeniable. Even though there are numerous synthetic drugs available, these days, there are issues with safety, efficiency, and unclear time settings for required outcomes with the current synthetic drug remedies available; therefore, there is growing attention to discovering alternative methods to fight hair loss, primarily through plant-derived formulations. While earlier reports mostly focused on screening compounds or plant extracts affecting 5α -reductase, our research takes a unique direction. We employed a biochemical and molecular biological approach by delving into the complicated biosynthetic pathways involving 17β -hydroxysteroid dehydrogenase (17β -HSD) and 3β -hydroxysteroid dehydrogenase (3β -HSD) in producing testosterone derived from cholesterol. This process conceded requiring experimental results, posing insights into the control of the testosterone/dihydrotestosterone (DHT) production pathway. Our study confirms a discovery platform for finding potential candidates as hair loss inhibitors, highlighting exploring various biochemical mechanisms involving 17β -HSD and 3β -HSD in combination with medicinal plant extracts.

Keywords: 17β -HSD; 3β -HSD; hair loss inhibition; plant extracts; human skin-based clinical studies



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1. Introduction

Recently, a resurgence of interest in herbal medicines and traditional remedies has resulted from the hunt for natural and practical solutions to hair loss. Researchers and enthusiasts have been exploring a broad range of medicinal plants to find potential natural therapies due to the desire for healthier and fuller hair. One of the most frequent trichinosis in clinical settings is alopecia, which has a profound effect on the self-confidence and persona of human beings. The universality of alopecia has expanded in recent years. Alopecia generally occurs due to a nutritional imbalance brought on by numerous factors, including food, alcohol, tobacco, environmental pollution, stress, repeated coloring, and permanent waves (perms) [1,2]. The age of patients with hair loss tends to be younger, where 40% of patients suffering from severe hair loss require relevant treatment [3,4].

Extrinsic factors include ultraviolet radiation, androgenic hormones, 5α -reductase activity-promoting substances, stress, diseases, and pollution [5,6]. It has been believed

that androgenic hormones and 5 α -reductase activity are the most significant causes of human alopecia, namely androgenic alopecia. Although it has not been considered a severe disease, several studies are still being carried out due to the growing public demand for hairdressing and beauty. In this regard, the motivation for hair growth is receiving more awareness in conventional and present-day medicine.

Dihydrotestosterone (DHT) stands out as the most potent hormone among the androgens, consistently recognized for its status as a pure androgen, unable to transform into estrogen. Derived directly from testosterone, DHT is acknowledged for its role in promoting hair loss. Given the well-documented involvement of 5 α -reductase in the metabolic conversion leading to DHT, extensive investigations into natural compounds that can impede this process have been undertaken. Minoxidil and finasteride are among the medications the U.S. Food and Drug Administration (FDA) has sanctioned as non-surgical therapy options for hair loss treatment [7]. Within the scalp, sulfotransferase transforms minoxidil into minoxidil sulfate, fostering hair follicular cell growth and staving off hair loss [8]. Finasteride, acting as a 5 α -reductase inhibitor, disrupts the conversion of testosterone into DHT, a key factor in alopecia disorders [9,10]. However, it is essential to note that these drugs carry significant side effects in some patients [11]. Consequently, the imperative to identify novel, safe, and effective treatments for hair loss is indispensable. Amidst this backdrop, the allure of harnessing the therapeutic potential of medicinal plant extracts has gained momentum. Therefore, finding novel, safe, and efficient medications to cure hair loss is indispensable.

Lately, interest has been observed in utilizing plant-based natural products or their extracts to prevent hair loss. Currently marketed as having natural components, it includes hair tonics, conditioners, cleansers, and growth promoters to cease hair loss [12]. It is well-accepted that most plants and their extracts carry countless components, for instance, flavonoids, polyphenols, terpenoids, carotenoids, and fatty acids, which boost the maintenance of human follicle health [13,14]. Since ancient times, plant-based formulations have been known for their safe properties and ease of acquisition from low-priced ingredients. While several people use natural products to prevent hair loss, little has been known about how they work. Various bioactive compounds in medicinal plants have long been valued for governing human physiological processes [15].

In the context of preventing hair loss, this study aims to conduct a scientific investigation of these botanical resources, i.e., plant products. By employing a complex screening-based procedure, our focus is to discover plant extracts that have the potential to be vital medicines against hair loss, contributing insight into their modes of action and their uses. An essential part in the biosynthesis of sex steroids is achieved through the 17 β -hydroxysteroid dehydrogenase (17 β -HSD) enzyme, which catalyzes the alteration of androstenedione (ASD) into testosterone and estrone into estradiol [16,17]. At the same time, 3-hydroxysteroid dehydrogenase-isomerase (3 β -HSD) is an enzyme necessary to produce testosterone [18,19].

With a target on 17 β -HSD or 3 β -HSD, we have examined the human biochemical pathway's components that induce testosterone/DHT to develop a novel screening platform for regulating hair loss. This approach finds medicinal plant extracts that have the potential to fight against hair loss by using our understanding of these biochemical pathways and aligns with the growing demand for all natural remedies by offering a holistic and sustainable alternative to current interventions. Throughout this study, we navigate the complexity of our screening process, outlining the norms for plant selection and experimental methods. Moreover, our investigation of mouse models enhance the safety, competence, and possible synergies amid plant compounds, supporting the literature on holistic hair care.

2. Material and Methods

2.1. Plant Extracts

The extracts were acquired from the Korea Research Institute of Bioscience and Biotechnology's (KRIBB) Overseas Biomaterials Center and the Korea Research Institute of Bio-

science and Biotechnology's Korean Plant Extract Bank. The potential extracts used in this work are listed in Table 1 below.

Table 1. List of plant extracts used in the experiments along with their source location and plant part used.

No.	Scientific Name	Plant Part Used	Source Location
1	<i>Cucurbita pepo</i> L.	Leaves, fruits, branches	Overseas Biomaterials Center
2	<i>Carthamus tinctorius</i> L.	-	Overseas Biomaterials Center
3	<i>Sophora flavescens</i> Ait.	Whole	Overseas Biomaterials Center
4	<i>Urtica dioica</i> L.	Twig	Overseas Biomaterials Center
5	<i>Rosmarinus officinalis</i> L.	-	Overseas Biomaterials Center
6	<i>Thymus vulgaris</i> L.	Leaves, stem, flower	Overseas Biomaterials Center
7	<i>Puerariae Flos</i>	-	Korean Plant Extract Bank
8	<i>Ginkgo biloba</i> L.	Leaves, stem	Overseas Biomaterials Center
9	<i>Aloe vera</i> (L.) Burm. f.	Leaves	Overseas Biomaterials Center
10	<i>Melissa officinalis</i> L.	Leaves	Overseas Biomaterials Center
11	<i>Ginkgo biloba</i> L.	Leaves, branches	Overseas Biomaterials Center
13	<i>Capsicum annuum</i> L.	Leaves	Korean Plant Extract Bank
14	<i>Camellia sinensis</i> L.	Leaves	Korean Plant Extract Bank
15	<i>Ginkgo biloba</i> L.	Leaves	Korean Plant Extract Bank
16	<i>Ginkgo biloba</i> L.	Leaves	Korean Plant Extract Bank
17	<i>Brassica oleracea</i>	-	China
18	<i>Crataegus pinnatifida</i> Bunge	Fruit	Korean Plant Extract Bank

2.2. Cell Culture

Human Follicle Dermal Papilla (hFDP) cells were purchased from Promocell, Heidelberg, Germany for the experiment. These cells were cultured in a follicle dermal papilla cell growth medium obtained from Promocell, Republic of Korea, and maintained in 5% CO₂, 37 °C in a humidified atmosphere. All the experiments were set up when the cells reached 70–80% confluency.

2.3. Cell Viability and Proliferation Assays

For the cell viability and proliferation assays, hFDP cells were seeded into a 96-well plate at a cell count of 1×10^4 cells per well one day before the experiment for cell attachment. Following 24 h (h), the cells were treated with various concentrations of extracts and incubated in a CO₂ incubator at 37 °C for the next 24 h. In this experiment, the WST assay (Ez-Cytox kit—DoGenBio, Seoul, Republic of Korea) was used to determine the cytotoxicity of the samples. Subsequently, a solution of 10% WST was added to each well, followed by incubation in an incubator (37 °C, 5% CO₂) for one hour. The samples were detected using a microplate reader (Biotek Synergy-HT, Winooski, VT, USA) to measure their absorbance at 450 nm. The experiment was conducted three times under the same conditions.

2.4. 17 β -Hydroxysteroid Dehydrogenase (17 β -HSD) Level

17 β -HSD activity was analyzed using the Human 17 β -hydroxysteroid dehydrogenase type 3 (HSD17b3) ELISA Kit (Biomatik, Kitchener, ON, Canada). All the ELISA steps were performed according to the protocols enclosed in the kit. In short, the cells were incubated according to the protocol after inoculating the cell lysate, which was treated with various extracts with different concentrations into the 6-well plate. After performing all the

intermediate steps, the 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution was added to each well and incubated further for 20 min in dark conditions. Following dispensing the stop solution, all the test samples were read at the wavelength deemed suitable in the protocol.

2.5. 3β -Hydroxysteroid Dehydrogenase (3β -HSD) Level

HFDP (3×10^5 cells/well) cells were seeded in 6-well plates, cultured for one day for the desired cell attachment, and treated with various extracts at various concentrations for 24 h. Following 24 h of the treatment, the cells were collected, and the lysates were prepared for the ELISA assays. The amount of 3β -HSD was measured using the Human HSD3B1 (Competitive EIA) ELISA Kit (LSBIO, Lynnwood, WA, USA) according to the manufacturer's instructions.

2.6. Determination of Androstenedione (ASD) Level

HFDP (3×10^5 cells/well) cells were seeded in 6-well plates, cultured for one day for the desired cell attachment, and treated with various extracts at various concentrations for 24 h. Following 24 h of the treatment, the cells were collected, and the lysates were prepared for the ELISA assays. According to the manufacturer's instructions, ASD was measured using the Human Androstenedione ELISA Kit (Biomatik, Cambridge, ON, Canada).

2.7. Clinical Trials

2.7.1. Donor Material and Information

This clinical study was conducted according to the Declaration of Helsinki principles and Korean Food and Drug Administration guidelines (IRB approval number-HM-IRB-P230292-1, HM-IRB-P23-0292-2). Hair testing was performed after written patient consent, and the ethics committee used the formulation mentioned in Table 2.

Table 2. Formulation (mixture) used in the clinical study.

Sample Extract	Concentration Ratio (1:1:2)
<i>Sophora flavescens</i> Ait.	1250 µg/mL (0.125%)
<i>Urtica dioica</i> L.	1250 µg/mL (0.125%)
<i>Ginkgo biloba</i> L.	2500 µg/mL (0.25%)

2.7.2. In Vivo Human Application Test

All the measurements and evaluations were conducted under constant temperature and humidity conditions (22 ± 2 °C, $50 \pm 5\%$), free from air movement and direct sunlight. The tests were conducted in a maintained space with the subject's skin resting.

Evaluation of Hair Root Volume Improvement Efficacy

In this test, the test subjects were divided into two groups; one group was designated as the test group using the test product, and the other group was defined as the control group using the control product. The root volume angle (°) was compared and analyzed using Image Pro 10 (Media Cybernetics, Inc., Rockville, MD, USA) after taking images of the top of the subject's head before and after four weeks of using the test product using a DSLR camera (Canon Inc., Tokyo, Japan).

$$\begin{aligned} & (\text{After 4 Weeks use/Before using test product} - 1) \times 100(\%) \\ & = \text{Individual improvement rate } (\%) \end{aligned}$$

Evaluation of Hair Elasticity Improvement Efficacy

MultiTest-dV (Memesis Ltd., West Sussex, UK) is a test device for tensile and compression and can measure a sample's load control, distance control, and tensile force. In this test, 20 test products and control products were applied to "40 human hair tresses,

where 20 were treated with the test product and 20 were treated with the control.” of 40 meters. MultiTest-dV was used to compare and measure the hair elasticity coefficient (MPa) of human stress before and after 4 weeks of test product use.

$$\begin{aligned} & (\text{After 4 Weeks use/Before using test product} - 1) \times 100(\%) \\ & = \text{Individual improvement rate } (\%) \end{aligned}$$

Evaluation of Scalp Sebum Reduction Efficacy

Sebumeter® SM815 (Courage + Khazaka GmbH, Köln, Germany) is a device that quantitatively analyzes the amount of sebum exuded by contacting the skin surface with a probe equipped with special paper using the optical reflection principle. The probe touches the skin surface with a force of 0.3 N for 30 s. Then, the device measures the amount of skin sebum. The measured amount of sebum is displayed in $\mu\text{g}/\text{cm}^2$ units through MPA software (version 1.1.5.0). In this test, the test subjects were divided into two groups: one group was designated as the test group using the test product, and the other group was assigned as the control group using the control product. Sebumeter® SM815 was used to compare and measure the amount of sebum ($\mu\text{g}/\text{cm}^2$) on the scalp before using the test product and after using it for 4 weeks.

$$\begin{aligned} & (1 - \text{After 4 Weeks use/Before using test product}) \times 100(\%) \\ & = \text{Individual improvement rate } (\%) \end{aligned}$$

Evaluation of Scalp Soothing Effect Efficacy

SkinColorCatch (Delfin Tech, Kuopio, Finland) is a device that measures skin color. White LED lamps corresponding to daylight are arranged in a circle inside the measurement chamber. When the device is brought into contact with the skin surface for measurement, the LED is illuminated at a 45-degree angle to the skin to minimize skin gloss, and the RGB color sensor detects the light reflected from the skin. Based on the measured RGB color, the CIE $L^*a^*b^*$ value, $L^*c^*h^*$ color, ITA degree, erythema index, and melanin index value are automatically calculated and displayed. In this test, the test subjects were divided into two groups: one group was designated as the test group using the test product, and the other group was defined as the control group using the control product. Using SkinColorCatch (Delfin Tech, Kuopio, Finland), the skin color redness (a^*) of the scalp area was compared and measured before using the test product and after using it for 4 weeks.

$$\begin{aligned} & (1 - \text{After 4 Weeks use/Before using test product}) \times 100(\%) \\ & = \text{Individual improvement rate } (\%) \end{aligned}$$

2.8. Statistics

All the values represent the mean \pm SD of the indicated number of replicates. Unpaired two-tailed Student's *t*-test or Dunnett's/Fishers-LSD multiple comparison tests with one/two-way ANOVA using PRISM version 10.0 software were used to determine the statistical significance, and the results are displayed as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, wherever applicable.

3. Results

3.1. Screening of the Medicinal Plant Extracts for Growth Activation

In our study, we first aimed to find effective plant materials for growth-promoting activity; therefore, we chose several plants, as indicated in Figure 1A, which may possess different active molecules and biological activities [20,21]. In addition, some medicinal plants have been revealed to be effective for burns to the skin, which suggests good cell proliferation activity [22]. Thus, we believe any plant material extract with cell proliferating activities might be a suitable hair growth agent. We screened 18 plant extracts in human dermal papilla cells in this view and determined the cell proliferation activity. To this end,

each extract was treated from lower to higher concentrations (0–400 $\mu\text{g/mL}$) in hFDP cells, and the effect was examined after 24 h. As a result of the cell proliferation rate in Figure 1B, samples 3, 4, 7, and 15 showed good results in the 100 $\mu\text{g/mL}$ range. However, 200 and 400 $\mu\text{g/mL}$ seemed to be lethal as shown by the cell viability results (Figure 1C). These results indicate that these four samples possibly influence dermal hair cell growth activity.

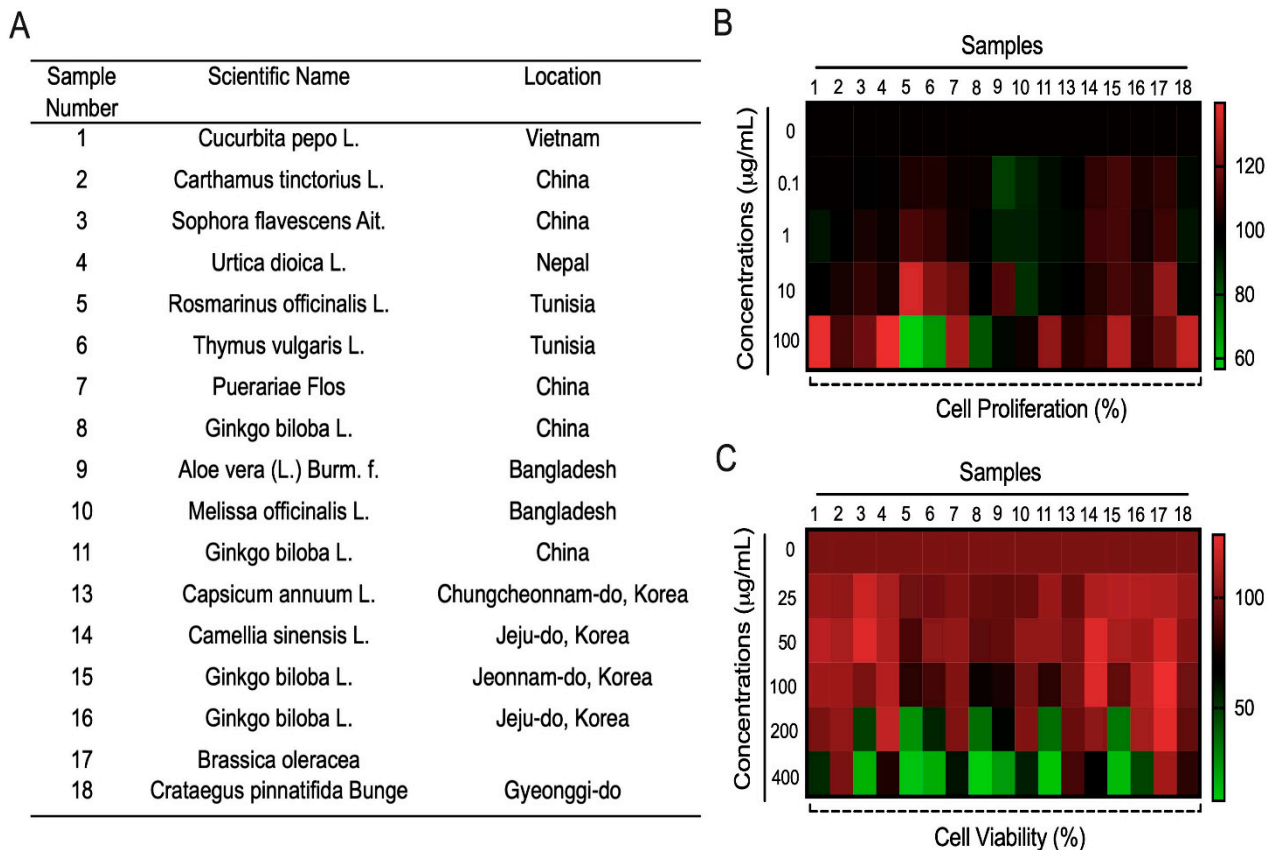


Figure 1. Outline of the medicinal plant extracts used and their cytotoxicity detection. (A) Representative table of the list of the medicinal plant extracts used in this study. (B) Determination of cell proliferation and (C) metabolic viability in human follicle dermal papilla (hFDP) cells following treatment with various extracts as indicated at 0–400 $\mu\text{g/mL}$ concentrations recorded at 24 h post-incubation.

3.2. 17 β -HSD Levels in Cultured hFDP Cells with Selected Plant Extracts

As described earlier, 17 β -HSD is necessary for the body's metabolism of steroid hormones [23]. Therefore, using the 17 β -HSD ELISA assay, we evaluated the activity of the 17 β -HSD enzyme in hFDP cultured cells with selected candidates that showed better proliferation rates, as depicted in Figure 1. As illustrated in Figure 2, *Sophora flavescens* Ait. (sample 3), *Urtica dioica* L. (sample 4), *Puerariae Flos* (sample 7), and *Ginkgo biloba* L. (sample 15) showed the most significant decrease in the 17 β -HSD enzyme up to 10 $\mu\text{g/mL}$. However, 100 $\mu\text{g/mL}$ seemed lethal in such activities. It is worth mentioning that the strongest effect was seen in *Ginkgo biloba* L., even at a higher concentration (100 $\mu\text{g/mL}$); however, *Puerariae Flos* failed to increase such responses after 0.1 $\mu\text{g/mL}$ (Figure 2A–D). These results suggest that the concentration can be varied depending on the extracts used on the dermal papilla cells.

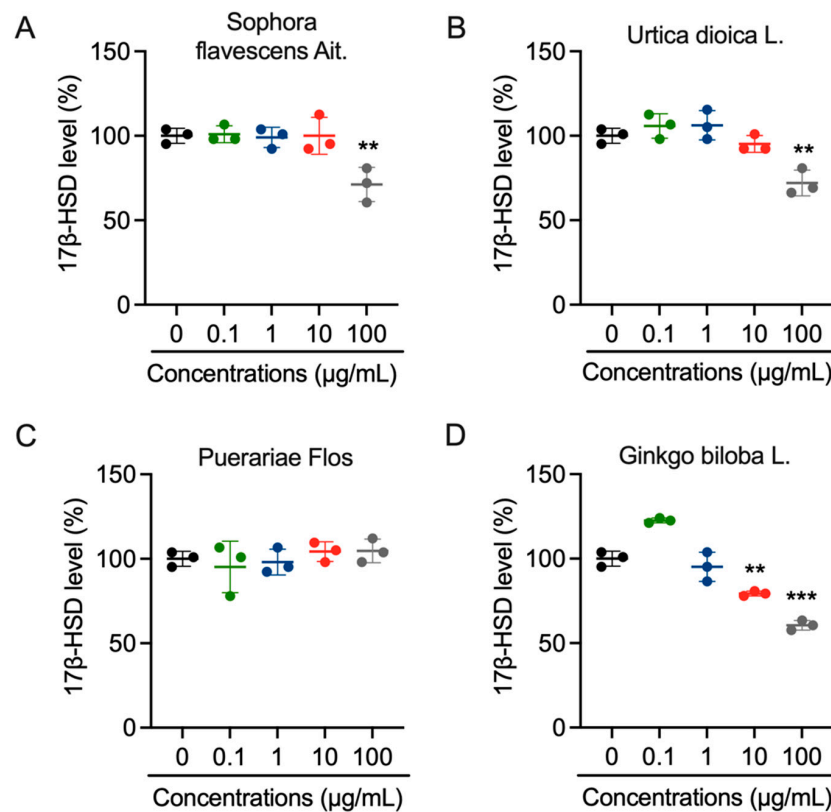


Figure 2. Assessment of 17-beta-hydroxysteroid dehydrogenase (17-β HSD) activity after exposure to plant extracts. (A–D) Determination of the 17-β HSD level in human follicle dermal papilla (hFDP) cells following treatment with *Sophora flavescens* Ait. (sample 3), *Urtica dioica* L. (sample 4), *Puerariae Flos* (sample 7), and *Ginkgo biloba* L. (sample 15) extracts at 0–100 μg/mL recorded at 24 h post-incubation. Dunnett's multiple comparison test with a one-way ANOVA was used to determine the statistical significance, and the results are displayed as ** $p < 0.01$ and *** $p < 0.001$.

3.3. Inhibition of 3β-HSD Level in Cultured hFDP Cells with Selected Plant Extracts

3β-HSD activity, identified by converting dehydroepiandrosterone (DHEA) to ASD, was investigated using the homogenate prepared from hFDP cells treated with plant extracts. In our results, we observed that *Sophora flavescens* Ait., *Urtica dioica* L., *Ginkgo biloba* L., and *Paeonia lactiflora* all showed no efficacy for the inhibition of the 3β-HSD enzyme even at higher concentrations (Figure 3A–D). Here, *Paeonia lactiflora* was included to compare the effect of selected plant candidates, as it has been suggested earlier that it treats alopecia through the inhibition of the steroid hormone pathway [24]. These results indicate that these extracts could not suppress the activity of the 3β-HSD enzyme during the conversion of ASD.

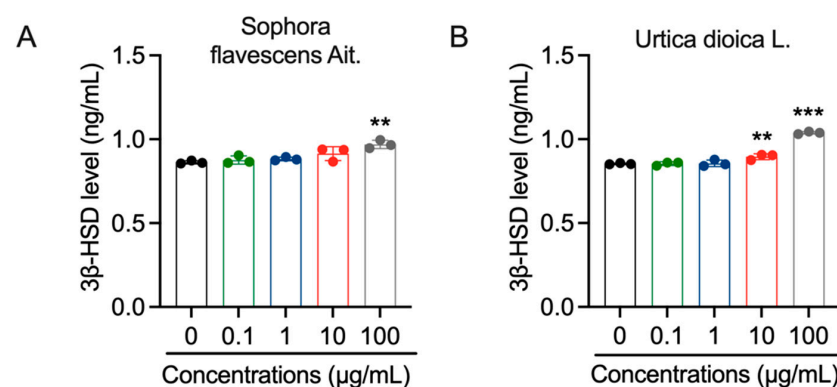


Figure 3. Cont.

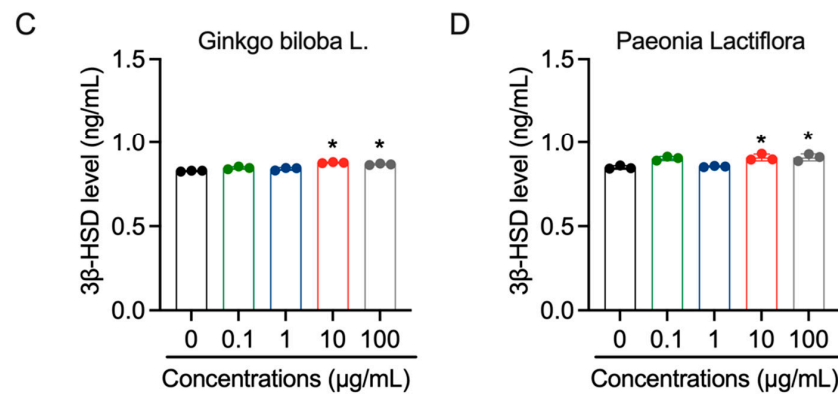


Figure 3. Assessment of hydroxy-delta-5-steroid dehydrogenase (3β-HSD) activity after exposure to plant extracts. (A–D) Evaluation of 3β-HSD I in human follicle dermal papilla (hFDP) cells following treatment with *Sophora flavescens* Ait., *Urtica dioica* L., *Ginkgo biloba* L., and *Paeonia lactiflora* plant extracts at different (0–100 μg/mL) concentrations recorded at 24 h post-incubation. Dunnett’s multiple comparison test with a one-way ANOVA was used to determine the statistical significance, and the results are displayed as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.4. Selected Plant Extracts Showed ASD Level Upregulation in Cultured hFDP Cells

To further understand the mechanism of hair loss, we examined the ASD levels in the plant extract-treated dermal papilla cells following 24 h of incubation. As Figure 4A–D shows, ASD enzyme concentrations were markedly increased when treated with *Sophora flavescens* Ait., *Urtica dioica* L., *Puerariae Flos*, and *Ginkgo biloba* L. Among all four tested plant extracts, *Urtica dioica* L. and *Ginkgo biloba* L. showed the maximum efficiency in the upregulation of the ASD levels in a concentration-dependent manner.

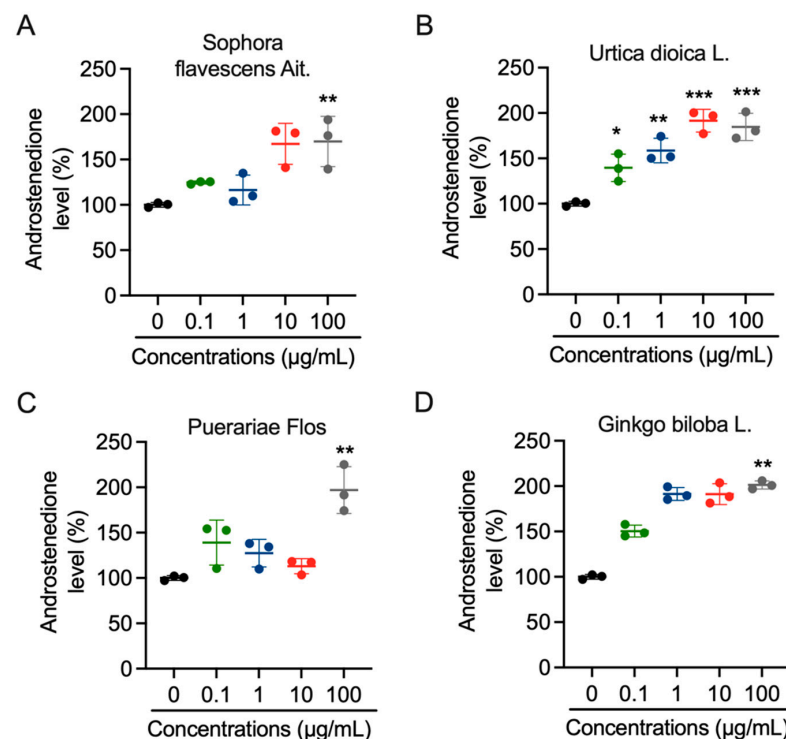


Figure 4. Assessment of ASD levels after exposure to plant extracts. (A–D) Evaluation of ASD activity in hFDP cells following treatment with *Sophora flavescens* Ait., *Urtica dioica* L., *Puerariae Flos*, and *Ginkgo biloba* L. extracts at different (0–100 μg/mL) concentrations recorded at 24 h post-incubation. Dunnett’s multiple comparison test with a one-way ANOVA was used to determine the statistical significance, and the results are displayed as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.5. Effect of Several Extract Mixtures in Cultured Follicle Dermal Papilla Cells

Finally, we wanted to test the effects of mixing the three extracts: *Sophora flavescens* Ait., *Urtica dioica* L., and *Ginkgo biloba* L., which had the greatest inhibitory effect among the experimental results. In this regard, the cytotoxicity of the four samples was examined by mixing the three extracts in ratios of 1:1:1, 2:1:1, 1:2:1, and 1:1:2 at 25 µg/mL and 50 µg/mL, and 100 µg/mL concentrations. The cell proliferation rate was validated in hFDP cells and compared to the untreated group by treating them with different concentrations. Notably, among the eight samples (Figure 5A), at 25 µg/mL, *Sophora flavescens* Ait. (sample 3), *Urtica dioica* L. (sample 4), and *Ginkgo biloba* L. (sample 15; Jeonnam Var.) showed the highest cell proliferation rate compared to the lower concentration of 2:1:1. These findings suggest that all four mixture samples showed no cytotoxicity in dermal papilla cells (Figure 5B).

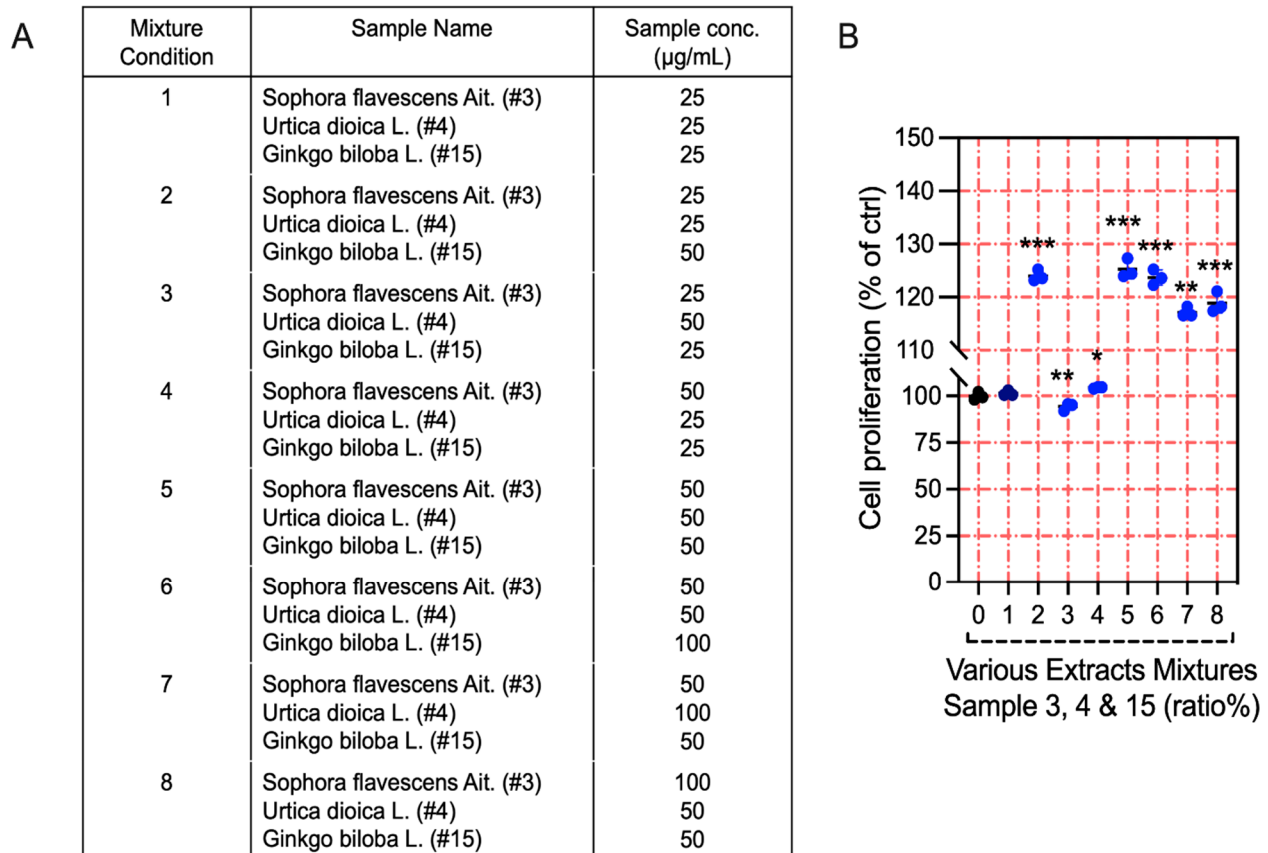


Figure 5. Testing the extract mixtures on human follicle dermal papilla cells. (A) The table indicates the mixtures used to evaluate the viability of the extract mixtures of *Sophora flavescens* Ait., *Urtica dioica* L., *Puerariae Flos*, and *Ginkgo biloba* L. at different concentrations ratios. (B) Determination of cell proliferation in human follicle dermal papilla (hFDP) cells following treatment with extract mixtures as indicated in panel A, and the cell viability was recorded at 24 h post-incubation. Dunnett's multiple comparison test with a one-way ANOVA was used to determine the statistical significance, and the results are displayed as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.6. Effect of Extract Mixtures on Direct Application on Human Scalps

Next, after testing the performance of individual plant extracts on human follicle dermal papilla cells, we opted to check their efficacy in human scalps using real-time experiments. To this end, we studied the hair root volume, serum reduction, scalp soothing effect, and hair elasticity tests. In the case of the control group, 24 test subjects who met the selection and exclusion criteria were recruited, and all test subjects completed the test typically, so the final 24 people were included in the result analysis. The average age of the test subjects was 50.1 years, the highest age was 61 years, and the lowest age was 40 years

(Table 3). On the other hand, the test group (extract mixtures) had 24 subjects who met the selection and exclusion criteria, and all the test subjects completed the test, so the final 24 people were included in the result analysis. The average age of the test subjects was 49.4 years, the highest age was 63 years, and the lowest age was 26 years (Table 4).

Table 3. Control group subject characteristics.

Subject	Category	Total Number of Respondents	Frequency (Number)	Percentage
Gender	Female	24	24	100.0%
	Male		0	0.0%
Age	20s	24	0	0.0%
	30s		0	0.0%
	40s		8	33.3%
	50s		15	62.5%
	60s		1	4.2%

Table 4. Test group subject characteristics.

Subject	Category	Total Number of Respondents	Frequency (Number)	Percentage
Gender	Female	24	24	100.0%
	Male		0	0.0%
Age	20s	24	1	4.2%
	30s		0	0.0%
	40s		12	50.0%
	50s		10	41.7%
	60s		1	4.2%

To evaluate the hair root volume compared to before using the test product, the root volume angle ($^{\circ}$) significantly increased in both the crown area of the test group and the crown area of the control group after 4 weeks of use. However, the increase in the root volume angle in the test group (mixture 2) was significantly higher than that in the control group (Figure 6A,B). The amount of sebum ($\mu\text{g}/\text{cm}^2$) in the sebum reduction experiments also decreased significantly in both the test and control group scalp areas after 4 weeks of use of the mixture product. However, the amount of sebum was considerably reduced in the mixture-treated group than in the control group (Figure 6C,D). After checking the reduction in scalp sebum, we intended to verify the scalp calming effect following our test product application. We observed skin color redness (a^*) was significantly reduced in both the test and control group scalp areas after 4 weeks of use, but interestingly, the amount of skin color redness reduction in the test (mixture 2) group was significantly higher than in the control group (Figure 6E,F). Finally, we were eager to check the hair elasticity following the test product application. Human hair's elasticity is a vital characteristic since it indicates its internal structure and is strongly associated with hair characteristics, such as smoothness and softness [25,26]. Our hair elasticity results demonstrate that the hair elastic modulus (MPa) of the test (mixture 2) group human hair tresses and the control group human hair tresses significantly increased after 4 weeks of use. Still, the increase in the hair elastic modulus of the test group was significantly higher than that of the control group (Figure 6G,H). During these test periods, no adverse reactions to allergenic or irritant contact dermatitis were reported or observed in the subjects after using the test product.

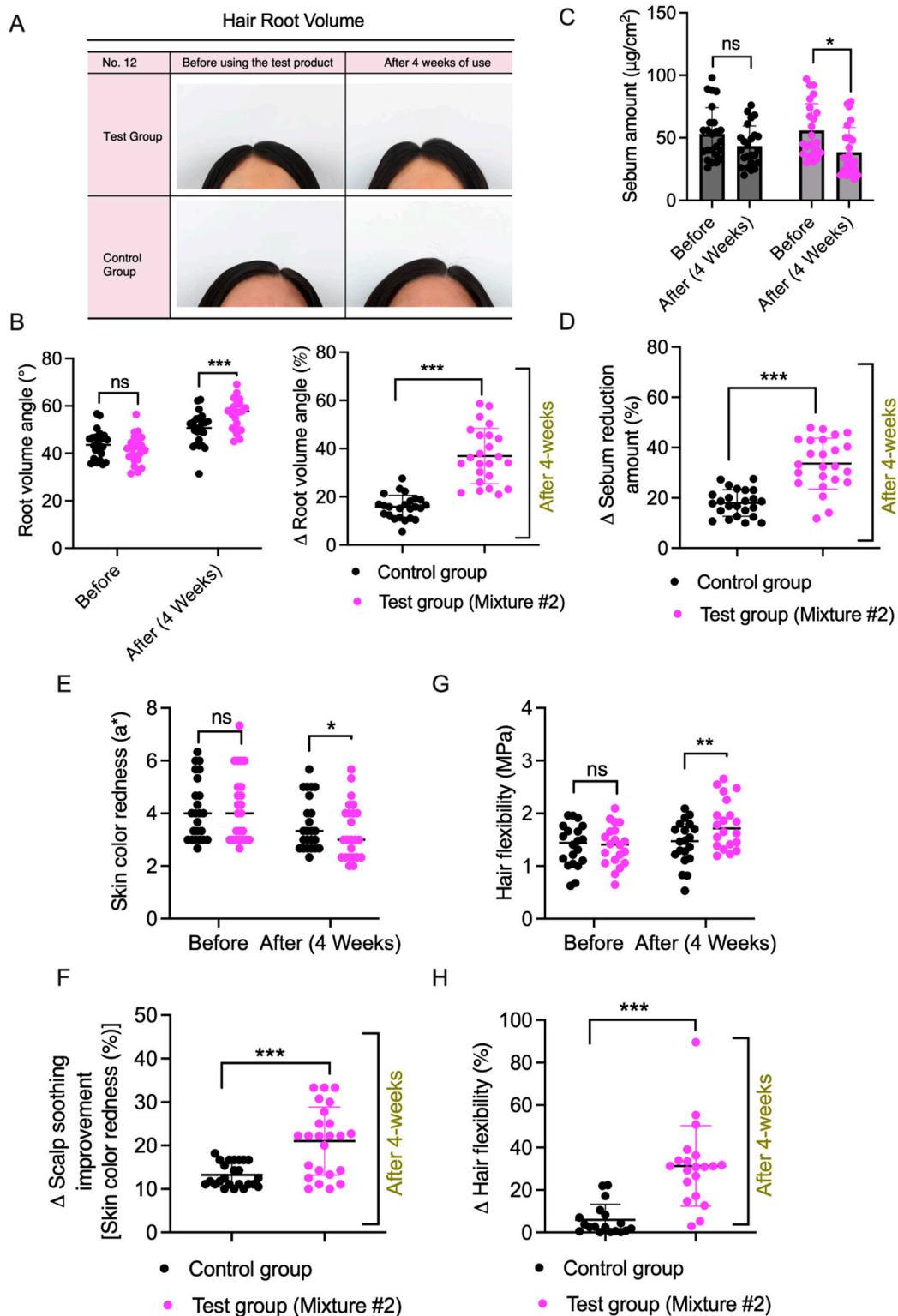


Figure 6. In vivo human skin application test. (A) Visual assessment results of the “test (mixture) product” group compared with those of the “control group”. (B) % Hair root volume angle recorded after 4 weeks of test product application. (C,D) The amount of sebum formation of the skin after application in human volunteers after 4 weeks post-application using the Sebumeter[®] SM815 device. (E,F) Scalp-soothing effect efficacy was measured using the SkinColorCatch device in human volunteers after 4 weeks post-application. (G,H) Measurement of the hair elasticity coefficient (MPa) in human stress to evaluate the hair elasticity in human volunteers after 4 weeks post-application. Unpaired *t*-test or Fisher’s LSD multiple comparison test with a two-way ANOVA was used to determine the statistical significance, and the results are displayed as * *p* < 0.05, ** *p* < 0.01, and *** *p* < 0.001.

4. Discussion

The hair follicle succeeds throughout the phases of growth (anagen), regression (catagen), and rest (telogen) in a continuous cycle and then revives in the subsequent hair cycle. Adjacent to the hair shaft's growth, the anagen phase is depicted by the visible proliferation of many epithelial sections within the hair follicle. The most proliferatively functional are the keratinocytes in the hair matrix surrounding the dermal papilla [27]. A substantial body of implicit data proposes that the dermal papilla, combined with specific fibroblasts detected at the source of the follicle, is fundamental in establishing the growth features of the hair follicle, and that it is influential in limiting the differentiation and multiplication of the hair follicle matrix [28].

The existing literature has validated that planted dermal papilla cells can produce diverse cytokine growth factors and other bioactive composites as bioassay supports. These combinations, whose accurate natures are unidentified so far, have a noteworthy impact on the proliferation of keratinocytes, outer root sheath cells, and other dermal papilla cells [29]. In light of such investigations, we conducted this study to examine if the chosen extracts can be used as hair loss prevention agents based on their cosmetological properties [30–44]. To achieve our goals, we evaluated the growth-proliferation abilities of these extracts using changes in the viability of human follicle dermal papilla cells. Interestingly, among all the tested samples, *Sophora flavescens* Ait. (sample 3), *Urtica dioica* L. (sample 4), *Puerariae Flos* (sample 7), and *Ginkgo biloba* L. (sample 15) showed a high proliferation rate in hFDP cells in tolerable concentrations (Figure 1).

It has been discovered that among the distinctive hormonal effects on hair development, androgens are a group that exceptionally stand out when studying alopecia. Though Aristotle first recognized the relation between androgens and male-pattern baldness, it wasn't until 1942 that Hamilton's studies, which intricated men who were operated on and subsequently lacking testicular androgens, decisively recognized the role of androgens—more precisely, testosterone or its metabolites—in the progression of public baldness [45].

In the process of androgen metabolism, a division of weak androgens known as 17-ketosteroids containing DHEA is described through a minimal affinity for the androgen receptor. These androgens can endure enzymatic transformation to more powerful forms with higher affinity for the androgen receptor, for instance, testosterone. The enzyme steroid 5 α -reductase metabolizes testosterone into DHT in numerous target tissues, which are implicated in the expansion of many ailments, including prostate cancer, acne vulgaris, and alopecia. The critical enzymatic pathways in converting weak androgens such as DHEA into further potent forms include 17 β -HSD, 3 β -HSD, and 5 α -reductase performance. In line with these agreements, our results indicate that *Sophora flavescens* Ait., *Urtica dioica* L., *Puerariae Flos*, and *Ginkgo biloba* L. can inhibit the activity of 17 β -HSD and 3 β -HSD enzymes.

Sophora flavescens, commonly called “Kushen” in Chinese medicine, is a conventional remedy evaluated for its miscellaneous medicinal properties, such as dropping fever, easing pain, substituting as an anthelmintic (against parasites), and helping stomach health [46,47]. It is established to include many prenylated flavonoids, containing sophoraflavanone G, kushenol E, kushenol B, kushenol L, kushenol M, kushenol N, kosamol A, norkurarinol, kurarinol, kushenol H, kuraridin, and kurarinone. Additionally, due to their free-radical scavenging and anti-aging attributes, *Urtica dioica* L. (nettle) extracts are utilized in cosmetic applications. They possess a mixture of reactive oxygen species scavengers, efficiently relieving free radical damage to the skin and thus demonstrating anti-aging impacts [48–50]. *Urtica dioica* extracts, such as ursolic acid and quercetin compounds, stand out as particularly intriguing for their promise in obtaining anti-aging properties. Ursolic acid, observed mainly in nettle roots, is documented as a prominent elastase inhibitor, contributing to its efficacy in anti-aging applications [49,51].

On the other hand, quercetin, primarily discovered in nettle leaves, appears as one of the leading antioxidants among the different compounds exhibited in the plant [52]. Apart from these two plants, our experiments revealed significant effects from another

plant—*Ginkgo biloba* L. This ancient medicinal tree species, with over 200 million years of history, is typically spread in regions such as Japan, China, and the Republic of Korea [53–55]. It is highly cultivated worldwide, with the valuable purpose of utilizing its leaves and seeds in food and health supplements [56]. Intriguingly, a finding was established by Japanese researchers a century before, where *Ginkgo biloba* showed promise in promoting hair growth on mice's shaved backs; however, the exact source in charge of this action is still unclear [43]. Among the active components in *Ginkgo biloba*, *Ginkgo biloba* polysaccharides have a considerable occurrence. Earlier reports indicated that the *Ginkgo biloba* polysaccharide displays significant immunomodulatory and anti-inflammatory effects [57]; nevertheless, whether it has an anti-hair loss property is rarely reported. Although current searches have researched the medicinal properties of its leaves, there remains a substantial gap in our insight into interpreting the molecular mechanism of action of the *Ginkgo biloba* extract.

To verify further, we looked at DHEA and ASD, which are androgenic hormones produced by the adrenal glands. These substances serve as precursors to the formation of estrogen and testosterone [58,59]. The conversion of ASD into testosterone occurs via the enzymatic activity of 17 β -HSD. Following this, the bloodstream carries testosterone, ultimately reaching its intended tissues and causing hair loss [60,61]. Therefore, it is further necessary to check the levels of ASD following the treatment of potential medicinal extracts used in this study to validate their possible effect in hair loss applications. Remarkably, there was a significant increase in ASD levels following the exposure of *Sophora flavescens* Ait., *Urtica dioica* L. and *Ginkgo biloba* L. extracts (Figure 4). Mixtures of these abstracts also showed considerable cell proliferation as observed in hFDP cells, suggesting their effectiveness towards real applications (Figure 5). Therefore, when we investigated the effect of these extracts (*Sophora flavescens* Ait., *Urtica dioica* L., and *Ginkgo biloba* L.) in human volunteers using clinical studies, these mixtures showed a significant improvement in hair root volume and elasticity, scalp soothing, and sebum reduction (Figure 6).

5. Conclusions

This study examined a novel possible mechanism of action of 18 medicinal herbs for hair loss treatment using an enzymatic activity inhibition approach. Considering the exceeding literature, the *Sophora flavescens* Ait., *Urtica dioica* L. and *Ginkgo biloba* plants and their derivatives have medicinal properties that can be applied as a natural source of bioactive molecules to be employed in various disorders. We showed that these extracts do not have the potential to inhibit the activity of 3 β -HSD but have the ability to block the formation of testosterone via 17 β -HSD, which consequently leads to hair loss inhibition. These results regarding targeting these enzymes and their related pathways of vital medicinal herbs associated with hair loss treatment offers an innovative foundation and helps to support further studies to enhance our understanding of the therapeutic mechanism of medicinal herbs for alopecia treatment. Our clinical studies on human volunteers recommend the possible application of these potential extracts for economical use; further investigations must be carried out using modern procedures emphasizing safety and clinical views.

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the clinical trial center. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Human Skin Clinical Trial Center Co., Ltd. (protocol code HM-IRB-P230292-1, HM-IRB-P230292-2, date of approval: 17 October 2023).

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