

Abstract

Equisetum debile is a local plant widely distributed in the highland area of Thailand, especially in the Northern part. The aims of the present study were to investigate the safety and anti-hair loss activity of *E. debile* extract in dermal papillary cells and develop prototype hair rejuvenation products from *E. debile* extract for commercial purposes.

The crude ethanolic extract of *E. debile* was used in the present study because of the highest yield of 16.13% and a good inhibitory activity against 5 α -reductase when compared to 1 mM ethinyl estradiol ($34.96 \pm 0.06\%$). The crude ethanolic extract was safe and showed no cytotoxicity on dermal papillary cells. It was noted that the viability of dermal papillary cells was decreased to $76.06 \pm 3.14\%$ after exposure to testosterone. Additionally, the cell viability was decreased to $68.98 \pm 7.14\%$ after exposure to testosterone and the crude ethanolic extract. There was no significant difference between the cells exposed and not exposed to the crude ethanolic extract. Therefore, the crude ethanolic extract was safe to the cell and not induced the cell death. However, the extract could not protect the cell death from testosterone. The crude ethanolic extract possessed inhibitory activity against interleukin-1 (IL-1), a cytokine that inhibit the dermal papillary cell growth, with the inhibition of $54.32 \pm 15.90\%$ at the concentration of 250 $\mu\text{g/ml}$. Therefore, the crude ethanolic extract has a promising approach to increase the dermal papillary cell growth.

Four hair serum products from the previous study, including formulation 3, 6, 9, and 10 were investigated for the satisfaction in 30 volunteers by using the questionnaires. The 5% minoxidil solution, was used as a control in this study. The results noted that formulation 10 was the best formulation that gained the highest satisfaction from the volunteers. Moreover, the satisfaction score was comparable to that of 5% minoxidil solution in the term of product odor, slippery hair, hair nourishment and anti-hair loss property. Therefore, formulation 10 was selected for the further performance test in volunteers. However, the product was adjusted to increase the consumer satisfaction by reducing the oily and greasy feeling and improve more desirable odor. Additionally, more active compounds were added into the formulation, including ginger extract, licorice extract, vitamin E, and wheat protein. Finally, the best modified formulation was formulation 10C.

To investigate the anti-hair loss efficacy, combing test was performed in 36 volunteers and number of hair falls after 30 times of combing was recorded. Hair serum formulation 10C significantly reduced the number of hair fall after 3 days of application ($P < 0.01$) and continue reducing tendency was obviously observed. The number of hair fall was reduced by $65.99 \pm 18.83\%$ after 4 weeks and up to $80.51 \pm 32.34\%$ after 12 weeks of application. Additionally, the hair of volunteers looks more dense, healthy, and shining. After 3 months of applications, the satisfactory of 36 volunteers were in a good level (3.89 ± 0.69).

In the present study, 21 shampoo base formulations were developed. Formulation 21 was the best formulation, followed by formulation 15, 9, and 6, respectively. Therefore, the best four formulations were selected for the further incorporation of the crude ethanolic extract in the concentration of 0.0125, 0.025, 0.05, and 0.1%w/w. It was noted that the viscosity of shampoo formulations increased after the crude ethanolic extract incorporation. The increased viscosity was obviously related to the amount of crude ethanolic extract. However, the color of shampoo formulation was darker after the Heating/Cooling stability test, especially the formulation with high content of crude ethanolic extract. Furthermore, the viscosity of formulation 9, 15, and 21 was changed after the Heating/Cooling stability test. Therefore, pearlescence agents were added into the shampoo formulations to solve the above problems. The results noted that the pearlescence agents could decrease the green color of the crude ethanolic extract and make more aesthetic appearance of the shampoo formulation. The viscosity of all formulations was adjusted after the addition of the pearlescence agents. The adjusted viscosity was in the range of 3.98 – 11.21 Pas. Moreover, the formulation could produce more foam after pearlescence agent addition. All pearlescence horsetail shampoo formulations were stable in the term of physical appearance, pH value, and viscosity after the Heating/Cooling stability test.

According to the hair revitalizing efficacy, the best horsetail shampoo was formulation 21, since it gave more noticeable hair shining than the others and no electrostatic was observed after combing with plastic comb. Moreover, the revitalizing efficacy of formulation 21 was comparable to the commercial anti-damaged hair shampoo. Besides, formulation 21 got the highest satisfaction score from 30 volunteers in the aspect of hair damage, split end, and brittle hair reduction. However, formulation 21 could produce small amount of foam. Therefore, the formulation was modified and formulation 21C was the best modified formulation. Therefore, formulation 21C would be an attractive formulation for damaged hair.

According to the consumer's satisfaction, formulation 9 got the highest satisfaction score from 30 volunteers among all formulations in the term of homogeneous appearance, appropriate viscosity, appropriate color, easiness to rinse off, cleanliness feeling, increase hair volume, slippery and soft hair, frizziness reduction, hair shining, and hair elasticity. However, formulation 9 could produce small amount of foam. Therefore, the formulation was modified and formulation 9D was the best modified formulation. Therefore, formulation 9D would be an attractive formulation for normal hair.

In conclusion, horsetail extract was attractive for hair care product development, including anti-hair loss serum and revitalizing shampoo. Additionally, these formulations had a potential to be manufactured in the further commercial aspects.