



# Effect of alpha-tocopheryl acetate, retinyl palmitate, and phytantriol on hair protection

Ki Young Ahn<sup>1,2</sup> · Hong Jong Song<sup>1,3</sup> · Dong Chung Kim<sup>1</sup>

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**Abstract** A hair essence containing  $\alpha$ -tocopheryl acetate, retinyl palmitate, and phytantriol (TRP-hair essence) was prepared. TRP-hair essence was excellent in thermal stability to the extent that it did not harden at all even at 210 °C. TRP-hair essence potently protected the hair from heat stress, significantly reducing the protein leakage in heat-treated hair ( $p < 0.001$ ). Treatment of TRP-hair essence to dyed human hair significantly protected hair against heat stress ( $p < 0.05$ ) as well as improved hair cuticle and color persistence ( $p < 0.05$ ). In addition, as a result of directly treating human hair with TRP-hair essence, the cuticle and tensile strength of human hair were significantly improved ( $p < 0.05$ ). These results suggest that TRP-hair essence can be effective for hair protection and hair quality improvement.

**Keywords** Hair protection · Phytantriol · Retinyl palmitate ·  $\alpha$ -Tocopheryl acetate · TRP-hair essence

## Introduction

Hair has gone beyond the simple function of head protection and body temperature regulation, and has become a means of expressing personal beauty and distinguishing oneself from others

[1]. Thermal and chemical treatments such as perm, dyeing, and hot air drying are required to make hair beautiful, which intensifies hair damage [2,3]. About 65-95% of hair is keratin protein which consists of 18 amino acids and cystine bridges [4]. Chemical treatments during perming and dyeing cause damage to keratin and cuticles. In addition, excessive or inappropriate use of heating devices such as hair irons and dryers causes severe hair damage such as protein denaturation and epidermal peeling [5,6].

Recently, various materials derived from natural sources have received great attention as substances that reduce hair damage and improve hair cuticles. In particular, vitamin E and A, typical fat-soluble antioxidant vitamins are known to be effective in improving skin and hair as cosmetic raw materials [7,8]. Alpha-tocopherol, a type of vitamin E, not only has antioxidant activity and protective effect against heat, but is also very stable enough to not decompose even when exposed to ultraviolet rays for 6 hours [7,9] thus being expected to be of great help to hair health. Vitamin A, also known as retinol and retinoid, is reported to be anti-wrinkle, improve skin and scalp, and promote hair growth [10,11] as an anti-aging ingredient. Among cosmetic raw materials other than vitamins, phytantriol which has been widely used as a hair cleanser not only increases the styling and conditioning effects of shampoo [12], but also improves the moisture retention of hair [13].

In addition,  $\alpha$ -tocopheryl acetate has been used as an alternative to  $\alpha$ -tocopherol itself because it is less acidic than  $\alpha$ -tocopherol and has a longer shelf life [14]. Retinyl palmitate, a more stable derivative than retinol, has been widely used in dermatological formulations because retinol is easily oxidized by heat and light [15]. Therefore, a hair essence containing  $\alpha$ -tocopheryl acetate, retinyl palmitate, and phytantriol (TRP-hair essence) is expected to contribute to maintaining and improving hair health due to its excellent physiological activities. In preliminary experiment, it was found that the hair essence with  $\alpha$ -tocopheryl acetate, retinyl palmitate, and phytantriol added at the same time was effective for hair protection. In this study, the efficacy of TRP-hair essence for preventing hair damage and improving cuticle was evaluated by

Dong Chung Kim (✉)  
E-mail: kimdc@chungwoon.ac.kr

<sup>1</sup>Departments of Chemical Engineering, Chungwoon University, Incheon 22100, Republic of Korea

<sup>2</sup>Seven Beauty Korea Company, Seoul 06112, Republic of Korea

<sup>3</sup>Gain Cosmetic Company, Incheon 21697, Republic of Korea

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measuring protein loss, cuticle, color, and tensile strength in human hair.

## Materials and Methods

### Materials

Human hair tresses were purchased from Morris & Co (Eumseung, Republic of Korea). Retinyl palmitate and phytantriol were from DSM (Amsterdam, Netherlands), and  $\alpha$ -tocopheryl acetate was bought from BASF (Berlin, Germany). Cyclopentasiloxane, dimethicone, disiloxane, cyclopentasiloxane/dimethicone, cyclomethicone/dimethiconol, and cyclopentasiloxane/cyclohexasiloxane/dimethicone/vinyl dimethicone crosspolymer were from KCC (Seoul, Republic of Korea). Bradford reagent and quick start bovine serum albumin standard were purchased from Bio-Rad (Hercules, CA, USA). Hair dye (LOMBOK) and shampoo (A3 MASTIC) were from Gain Cosmetic (Incheon, Republic of Korea). Other chemicals were of analytical grade.

### Preparation of TRP-hair essence

TRP-hair essences were prepared as shown in Table 1. All process was carried out at room temperature by a disper mixer (Sunjin Tech, Incheon, Republic of Korea). After cyclopentasiloxane and cyclopentasiloxane/cyclohexasiloxane/dimethicone/vinyl dimethicone crosspolymer were mixed, cyclopentasiloxane/dimethicone and cyclomethicone/dimethiconol were added and suspended. And then dimethicone, disiloxane, *Opuntia ficus-indica* extract, oil complex, cysteine complex, fragrance, phytantriol,  $\alpha$ -tocopheryl acetate, and retinyl palmitate were added and finally suspended.

### Thermal stability of TRP-hair essence

In TRP-hair essence,  $\alpha$ -tocopheryl acetate, retinyl palmitate, and phytantriol were added in the same amount in the range of 0.005 to 0.02% (w/w). The negative control, positive control and experimental hair essences shown in Table 1 were each heated to 210 °C for 2 min on a hair straightener (WorldJB, Tokyo, Japan). The difference in fluidity according to the TRP content was visually observed and shown as photographs.

### Protection effect of TRP-hair essence against thermal damage of hair *in vitro*

To confirm the *in vitro* effect of TRP-hair essence on the degree of hair damage and protein loss caused by heat treatment, 0.2 g of human hair tresses was treated with 500  $\mu$ g of the positive control and of experimental hair essences, respectively, and left at room temperature for 10 min. Thereafter, the treated hair samples were washed three times with distilled water and then treated in a hot oven at 150 °C for 20 min. The damaged hair samples were immersed in 2 mL of 2.5% NaOH solution for 30 min to elute the protein, and then centrifuged at 3,000 g-force for 5 min to obtain a supernatant [16,17]. Proteins in the supernatant were quantified based on the Bradford method [18]. The total amount of the leaked protein from the hair was expressed as the equivalent of bovine serum albumin.

### IRB approval

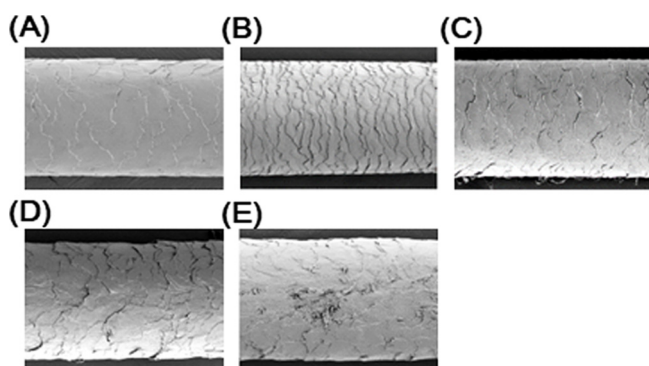
All experiments investigating the effects of TRP-hair essence on human hair were approved by the Institutional Review Board of KC Skin Clinical Research Center (Seoul, Republic of Korea) with approval number 1-70005235-A-N-01-201704-HR-KC-170501-C1-02 and 1-70005235-A-N-01-201803-HR-KC-180402-C2-02. Twenty female subjects, which were healthy person aged 19 to 59 years old with a hair length of 10 cm or more, were invited to evaluate the color persistence and tensile strength of hair, and the improving degree of hair cuticle.

### Effects of TRP-hair essence on color persistence and cuticle protection of dyed human hair *ex vivo*

To confirm the *ex vivo* effect of TRP-hair essence on the color persistence of dyed hair, 40 tresses of human hair were prepared from the female subjects, and dyed with LOMBOK dye. The dyed hair tresses were divided into two groups, and then were treated by positive control and experimental hair essences, respectively. Brightness values were measured using the Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) before use of each hair essence, immediately after use of each hair essence, after shampooing 7 times of each hair essence-treated hair, and after shampooing 14 times of each hair essence-treated hair, respectively.

**Table 1** Ingredients of negative control, positive control, and TRP-hair essence

Hair essences	Ingredients (%)
Negative control	The negative control means that no hair essence was treated.
Positive control	Cyclopentasiloxane (33.098), cyclopentasiloxane/dimethicone (25.000), cyclomethicone/dimethiconol (20.000), dimethicone (10.000), disiloxane (8.000), cyclopentasiloxane/cyclohexasiloxane/dimethicone/vinyl dimethicone crosspolymer (3.500), <i>Opuntia ficus-indica</i> extract (0.001), oil complex (0.001), cysteine complex (0.100), fragrance (0.300)
Experimental (TRP-hair essence)	Cyclopentasiloxane (33.083~33.038), $\alpha$ -tocopheryl acetate (0.005~0.020), retinyl palmitate (0.005~0.020), phytantriol (0.005~0.020), The content of cyclopentasiloxane was reduced as much as phytantriol, $\alpha$ -tocopheryl acetate, and retinyl palmitate was added. The rest consisted of the same ingredients and contents as above positive control.



**Fig. 1** Grades of hair surface damage analyzed by scanning electron microscopy. (A) Very healthy hair, grade 1; (B) Healthy hair, grade 2; (C) Common hair, grade 3; (D) Damaged hair, grade 4; (E), Very damaged hair, grade 5

To confirm the protecting effect of TRP-hair essence against heat stress *ex vivo*, 40 tresses of human hair were prepared from the female subjects, and dyed with LOMBOK dye. The dyed hair tresses were divided into two groups, and then were treated by positive control and experimental hair essences, respectively. The hair tresses treated with each hair essence were washed with shampoo and distilled water, and then subjected to heat damage with a hair dryer, respectively. After two types of hair essence were applied to human hair tresses once a day for 2 weeks, respectively, heat stress was repeated with a hair dryer to apply to the hair. The human hairs were photographed with a scanning electron microscope [19] before use of each hair essence, after one application of each hair essence treatment and heat stress, and after 14 times application of each hair essence treatment and heat stress. The degree of damage to the surface of the photographed hair cuticle was evaluated by grade as shown in Fig. 1.

**Effects of TRP-hair essence on tensile strength and cuticle of human hair**

Hairs before and after treatment of TRP-hair essence were collected from female subjects, and then their tensile strength was measured using Neo-Tribo FCMS-170 (Neoplus, Daejeon, Republic of Korea), respectively [20].

To investigate the effect of TRP-hair essence on improving the

hair cuticle in female subjects, TRP-hair essence-treated and untreated hairs were subjected to heat stress at 150 °C for 2 min using a hair straightener, respectively. After washing both hairs using shampoo and distilled water, the damaged hair samples were collected from female subjects, and then photographed using a scanning electron microscope [19]. The grade of hair before and after heat treatment and TRP-hair essence treatment was determined by evaluating the degree of damage to the surface of the hair cuticle photographed as shown in Fig. 1.

**Statistical analysis**


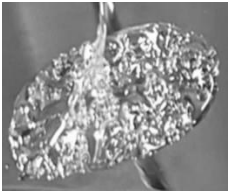

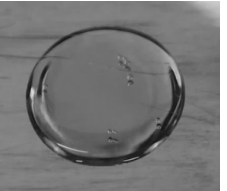
Data were analyzed by using SPSS Statistics 23.0 for Windows (IBM, Armonk, NY, USA). A *p*-value less than 0.05 (typically *p* < 0.05) is statistically significant. When comparing immediately after using TRP-hair essence and 24 h later, if the normality test was satisfied, a parametric repeated ANOVA test was performed, and if not, a nonparametric Friedman test was performed. When comparing the non-use and use of TRP-hair essence, if the normality test was satisfied, a parametric independent t-test was performed, and if not, a nonparametric Mann-Whitney U-test was performed. In addition, when comparing before and after using TRP-hair essence, if the normality test was satisfied, a parametric paired samples t-test was performed, and if not, a nonparametric Wilcoxon signed ranks test was performed.

**Results and Discussion**

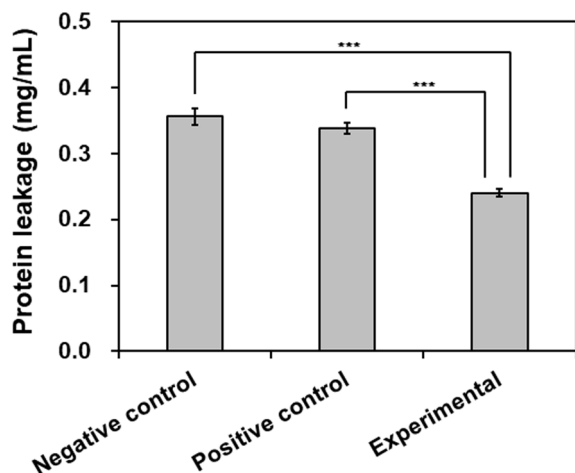
**Thermal stability of TRP-hair essence**

When a hair essence without TRP was placed on a hair straightener at 210 °C, it was completely hardened to the point that it could not be used. But the hair essence to which 0.01 and 0.02% (w/w) of each component of TRP was added did not harden at all (Table 2). Therefore, it was possible to significantly improve the thermal stability of the hair essence by adding only 0.01% (w/w) of each component of TRP. It is known that the hair protection effect of hair essence decreases sharply when hair treated with a general hair essence is subjected to heat stress at 180 °C or higher using a hair straightener [21]. In this study, TRP is very effective in maintaining the properties of hair essence even at high

**Table 2** Fluidity degree of hair essences according to TRP content at 210 °C

Each TRP content <sup>a</sup>	0%	0.005%	0.01%	0.02%
Degree of fluidity				
	-	+	++	++

<sup>a</sup>TRP-hair essences contained the same content of α-tocopheryl acetate, retinyl palmitate, and phytantriol, relatively



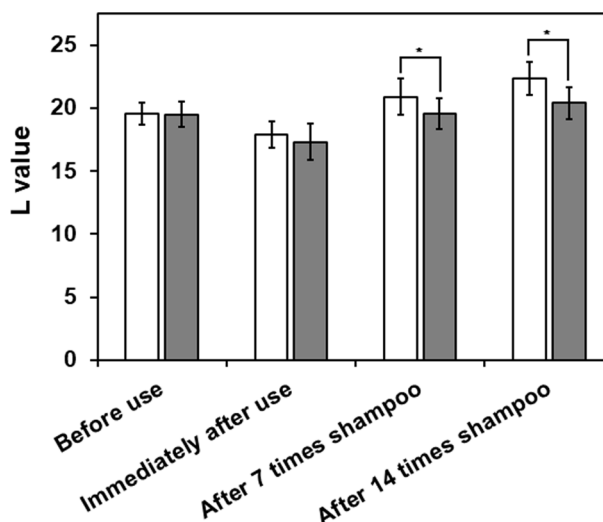
**Fig. 2** Amount of protein leaked after thermal damage to hair treated with TRP-free hair essence (positive control) and TRP-hair essence (experimental). A case not treated with hair essence was expressed as a negative control. \*\*\* $p < 0.001$  by independent t-test

temperatures, so it is expected that the hair protection effect of TRP-hair essence would be excellent. Alpha-tocopheryl acetate and phytantriol are known to have excellent thermal stability. Alpha-tocopheryl acetate is thermally stable up to 240 °C [22], and phytantriol boils over 300 °C at atmospheric pressure [23]. The thermal stability of TRP-hair essence is considered to be mainly due to  $\alpha$ -tocopheryl acetate and phytantriol.

#### Protection effect of TRP-hair essence against thermal damage of hair *in vitro*

After treating human hair with negative control, positive control, and experimental hair essences, respectively, the effect of preventing hair protein leakage caused by heat damage was confirmed (Fig. 2). The amount of protein leakage was  $0.356 \pm 0.012$  mg/mL in the negative control group not treated with hair essence, and  $0.338 \pm 0.008$  mg/mL in the positive control group treated with general hair essence, respectively. The amount of protein leakage in the experimental group treated with TRP-hair essence was  $0.240 \pm 0.006$  mg/mL, which was significantly lower than that of the negative and positive control groups ( $p < 0.001$ ). This showed that TRP-hair essence effectively protects the hair cuticle from heat damage, thus significantly reducing protein leakage from the hair.

Even if only heat of 40-70 °C is applied to the hair, the epidermis layer falls off and the cortex is exposed [24]. Heat treatment is essential for dyeing and perming, and it is common that hair protein loss increases proportionally as the temperature rises during dyeing and perming [25]. There was no significant difference in protein leakage between the negative control and the positive control groups. However, even after heat stress at 150 °C, the TRP-hair essence-treated hair showed a significant reduction in protein leakage by 32.61% ( $p < 0.001$ ) compared to the negative



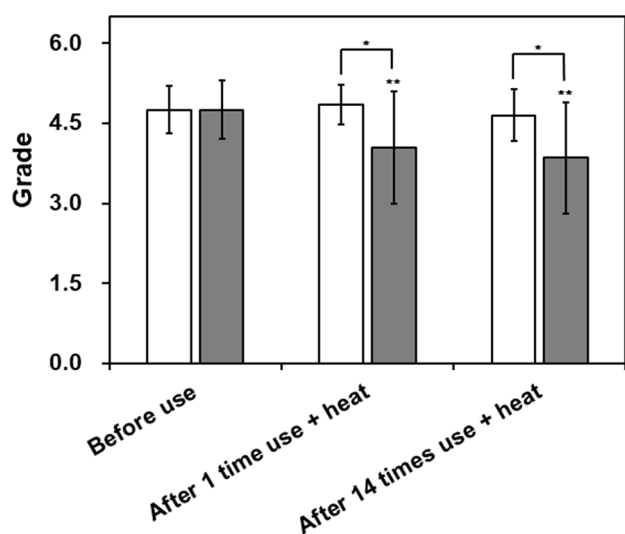
**Fig. 3** Effect of TRP-free hair essence (positive control, □) and TRP-hair essence (experimental, ■) on the durability of dyed hair. The L value of hair was measured before their use, immediately after their use, after shampooing 7 times of each hair essence treated hair, and after shampooing 14 times of each hair essence treated hair, respectively. \* $p < 0.05$  by independent t-test

control group, indicating that TRP hair essence had an excellent hair protection effect against heat damage.

#### Effects of TRP-hair essence on color persistence and cuticle protection of dyed human hair *ex vivo*

As a result of comparing the color persistence of dyed hair treated with the positive control and the experimental hair essences (Fig. 3), the color persistence of the dyed hair treated with TRP-hair essence was significantly superior to that of the dyed hair treated with positive control ( $p < 0.05$ ). Even after shampooing 14 times, the dyed hair treated with TRP-hair essence maintained color better than the hair treated with positive control ( $p < 0.05$ ). Among natural plant oils, green tea seed oil, which has a high content of unsaturated fatty acids, is known as a very good natural hair treatment agent [6]. In this study, the TRP-hair essence was as effective as green tea seed oil in maintaining the color of dyed hair. Because the maintenance period of dyed hair is usually about 1 month, the dyed hair rapidly discolors after shampooing 35 times [26]. TRP-hair essence could help a lot in prolonging the color persistence of dyed hair.

After treating the dyed hair with a combination of hair essence and heat stress once a day for 2 weeks, the degree of damage to the hair cuticle was evaluated using a scanning electron microscope (Fig. 4). Even when the hair essence and heat treatment were applied 14 times, the cuticle of the hair treated with TRP-hair essence was improved by 18.25% ( $p < 0.05$ ), whereas the cuticle of the hair treated with positive control had little improvement effect. TRP-hair essence not only protects the hair cuticles from heat stress caused by the use of a hair dryer, but also improves the



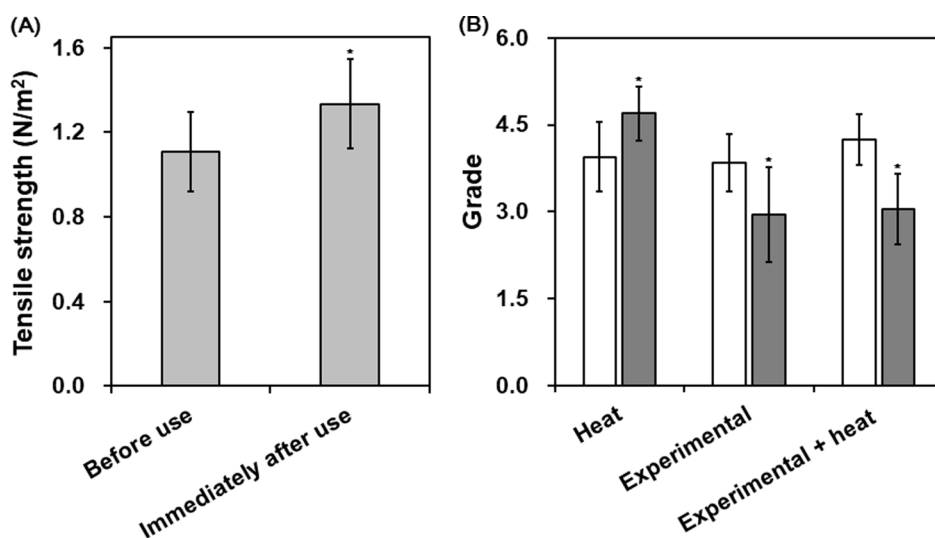
**Fig. 4** Protection effect of TRP-free hair essence (positive control, □) and TRP-hair essence (experimental, ■) against hair damage caused by heat stress. The grade of hair was measured before use, after one application of treatment and heat stress, and after 14 times application of treatment and heat stress, respectively. \*\* $p < 0.017(5\%/3)$  by Friedman test, post hoc Wilcoxon signed rank test, \* $p < 0.05$  by independent t-test

quality of the cuticles. Since hair is very weak to heat and easily damaged by UV rays, heat stress and UV exposure cause moisture removal from hair and loss of hair elasticity [27,28]. In this study, TRP-hair essence protected the hair cuticles even after repeated heat treatment and shampooing, thus being able to be applied as an excellent hair protectant.

### Effects of TRP-hair essence on tensile strength and cuticle in human hair

As a result of comparing the tensile strength of the hair before and immediately after TRP-hair essence treatment (Fig. 5A), the tensile strength significantly increased to  $1.335 \pm 0.211 \text{ N/m}^2$  immediately after TRP-hair essence treatment compared to  $1.107 \pm 0.189 \text{ N/m}^2$  before TRP-hair essence treatment ( $p < 0.05$ ). It was reported that the addition of Pura powder during permanent wave treatment increases the tensile strength of hair [29], and keratin peptide has the effect of improving the tensile strength and elasticity of hair [30]. The tensile strength of hair using shampoo containing ethanol extracts of black bean, rice bran and wheat increased by ~40% compared to before its use [20]. Compared with the 168% increase in hair tensile strength by treatment with *Cordyceps militaris* extract containing a large amount of essential amino acids for 70 days [19], the hair tensile strength was improved by 23.23% with just one treatment of TRP-hair essence. It has been shown that TRP-hair essence has an excellent effect on increasing the tensile strength of hair.

As a result of evaluating the degree of hair damage before and after heat stress to human hair (Fig. 5B), the heat stress applied without treatment of hair essence increased hair damage by 20.42% ( $p < 0.05$ ). However, TRP-hair essence treatment improved the hair cuticle by 28.25% ( $p < 0.05$ ) in spite of applying heat stress to the hair. Even when hair is treated with general hair essences, severe damage such as epidermal abrasion and hair thinning commonly occurs depending on the temperature and the number of heat treatments [21,31]. In this study, TRP-hair essence showed the excellent effect of protecting as well as improving the hair cuticle.



**Fig. 5** (A) Effect of TRP-hair essence on hair tensile strength. The tensile strength of hair was measured before and immediately after use of TRP-hair essence, respectively. \* $p < 0.05$  by paired samples t-test. (B) Protection effect of TRP-hair essence on hair cuticle. The grade of hair was measured before (□) and after (■) treatment with heat and TRP-hair essence respectively and simultaneously. \* $p < 0.05$  by Wilcoxon signed ranks test

Phytantriol has been used in about one hundred of cosmetic products at concentrations ranging from 0.0002 to 1.0% [23] because it improves the moisture retention of hair and skin [13]. Tocopheryl acetate is known to be effective in maintaining hair and scalp health by reducing the oxidative stress that causes scalp destruction [32]. Certain vitamin A derivatives like retinyl palmitate have been reported to be beneficial to both scalp and hair [33]. Therefore, the protective effect of TRP-hair essence on hair is considered to be due to the combinatorial use of  $\alpha$ -tocopheryl acetate, retinyl palmitate, and phytantriol. In conclusion, TRP-hair essence was thermally stable, and prevented the leakage of hair protein by protecting the hair cuticles from heat stress, and improved the tensile strength and the cuticles of human hair as well.

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