

Original Article

GREEN TEA (*CAMELLIA SINENSIS*, L.) ETHANOLIC EXTRACT AS HAIR TONIC IN
NUTRACEUTICAL: PHYSICAL STABILITY, HAIR GROWTH ACTIVITY ON RATS, AND SAFETY
TEST

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ABSTRACT

Objective: Herbal and synthetic cosmetic products have been developed to unravel problem of hair loss, yet synthetics are potential to give side effects (e.g. local irritation), whilst herbal products are generally safer. Green tea is one of food derived active ingredient potential as topical hair grower. The purpose of this study is to formulate nutraceutical hair tonic(s) which is stable, effective towards hair growth, and safe.

Methods: Ethanolic green tea extract (GTE) was formulated into varied concentrations i.e. 2.5%, 5%, and 7.5%. Physical stability test performed was cycling test, storage in high temperature ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$), room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), and low temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$). Activity of hair growth test was conducted by hair length measurements on day 7, 14, and 21, plus diameter measurements and total weights of hair on day 21. Safety test was carried out on 9 volunteers' upper hands.

Results: Results showed the hair tonic was stable in storage, except in low temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$). In addition to giving hair growth activity, all of the formulas had greater activity than synthetic drug i.e. minoxidil 2.5%. These hair tonics were safe and did not irritate skin.

Conclusion: The most optimal formulation was formula 1 with GTE concentration of 2.5%.

Keywords: Activity towards hair growth, Hair tonic, Green tea, Physical stability test, Safety test.

INTRODUCTION

By crowning and completing the whole physical appearance, hair presents important roles whether in men or women. Hair promotes a statement of personal physical character with its length, color, and style; therefore hair oftenly considered as nonverbal expression of personality [1]. However, in case of physical condition, emotional stress or physical stress, nutrient deficiency, hormonal disturbances, or particular drugs, hair loss could occur to men or women in all ages [2]. As the frequency and quantity increased, this problem of hair loss which eventually ends to baldness has become a trending common problem causing anxiety and stress to every individual [3].

Various cosmetic products had been developed to solve the problem of hair loss as well as baldness, either from synthetic or natural products. However, synthetics, i.e. minoxidil, are more potential to cause side effects (pruritus, local irritation, hypertrichosis, headache, etc.) [4][5][6]. Recently, trend to use food ingredients in cosmetic products has emerged since the demand for products that are safer and free from synthetics are high [7]. Hence, natural products such as nutraceuticals which origin's are from food, is one of alternative solution, providing lower chance of side effects.

The word nutraceutical was derived from "nutrition" and "pharmaceutical", thus nutraceutical was defined as food or part of food that gives pharmacology advantages for health or medical, including prevention and treatments of diseases [7][9][10]. The emerged trend in which food ingredients are used in cosmetics showed that nutraceuticals are not only strictly for oral, but also for topical preparations. In such opportunity, public education and socialization of this product would be more acceptable in society [7]. Green tea leaves (*Camellia sinensis*, L.), are one of this potential nutraceuticals. As green tea has been consumed since early age as safe food, it showed no side effects found by consuming green tea in long time [11]. Moreover, subacute toxicity research on green tea proofed no side effects shown [12]. Green tea (*Camellia sinensis*, L.) has been well-known in its daily use as food, as well as nutraceutical by utilizing its pharmacological effect of antioxidant, even anticancer [12][13]. Remarkably, unlike its common use, green tea also showed a great potential for its pharmacology activity

towards hair growth. Esfandiari & Kelly (2005) showed in 50% of green tea polyphenol extract fraction, administered orally to black rats which are having spontaneous hair fall, resulting 33% rats showed significant hair growth in 6 months [14]. Further explanation showed by Kim et al. (2007), which stated an increase of ex vivo and in vivo human hair growth activity from green tea epigallocatechin-3-gallat (EGCG) [15]. This research explained the mechanism of hair growth from EGCG was by stimulating cell proliferation and anti-apoptosis effect on hair dermal papilla cell. Some researchers, Asyarie, Immaculata, & Triwahyuni (2007) [16], and Lindawati, Dhurhanian, & Murtisiwi (2011) [17] also have tried to formulate this green tea in gel dosage form and evaluate its activity towards hair growth. This showed how green tea is potential as hair growth enhancer, thus the development of green tea extract as hair growth enhancer should be keep continued.

In this research, green tea extract (GTE) is formulated in hair tonic dosage forms as nutraceutical topical preparation. Then, this research will evaluate GTE hair tonic's stability, hair growth activity on rats comparing to synthetic drug (i.e. minoxidil), and safety. As hair tonic is formed in liquid, it provides ease and convenient application to hair skin, great trans-appendageal absorption and easily dispersed, not greasy and does not leave any residue on hair skin that can induce hair scalps. Subsequent evaluations after formulating hair tonics were performed to define the pharmacology activity, stability, and safety.

MATERIALS AND METHODS

Materials

Ethanolic green tea extract 70% (Bogor, Indonesia), methyl paraben, disodium edetat, sodium metabisulfite, menthol, propylene glycol, ethanol 96% (obtained from PT Brataco Chemika, Indonesia), minoxidil (obtained from Fidia Farmaceutici, Indonesia), aquadest and depilatory cream *Veet*® (PT Reckitt Benckiser, Indonesia).

Animals

Animals used in this research are healthy *Sprague-Dawley* male rats, aged 7-8 weeks, and weighed 150-200 gr (Institut Pertanian Bogor,

Indonesia). All animal laboratory experiments were ethically approved by The Ethics Committee of the Faculty of Medicine, University of Indonesia, Indonesia.

Methods

Formulation and Preparation of Green Tea Hair Tonic

All materials and apparatus were prepared previously. Sodium metabisulfite and disodium edta were dissolved in 3.5 ml aquadest,

and mixed with dissolved tween 80 in the remaining aquadest. Methyl paraben was dissolved in 2 ml ethanol, menthol in 3 ml ethanol, and GTE in adequate volume of ethanol. Water and alcohol mixture were mixed gradually then added to mixture of propylene glycol and ethanol gradually while stirring until homogenous.

Evaluation of Hair Tonic Dosage Form

In this research, evaluations of hair tonics conducted were organoleptic, homogeneity, pH, consistency, viscosity, density [19].

Table 1: It shows formulation for GTE Hair Tonic (Formula 1, 2, 3), negative control, and positive control minoxidil

Materials	Quantity (%) (w/w)				
	Negative Control	F1 (%)	F2 (%)	F3 (%)	Positive Control
Green Tea Extract	-	2.50	5.00	7.50	-
Minoxidil	-	-	-	-	2.50
Ethanol 96%	75	75	75	75	75
Propylene glycol	10	10	10	10	10
Tween 80	1.00	1.00	1.00	1.00	1.00
Methyl Paraben	0.075	0.075	0.075	0.075	0.075
Na ₂ EDTA	0.025	0.025	0.025	0.025	0.025
Menthol	0.1	0.1	0.1	0.1	0.1
Sodium Metabisulfite	0.2	0.2	0.2	0.2	0.2
Aquadest	13.6	11.1	8.6	6.1	11.1

Physical Stability Test

Physical evaluation of hair tonics performed such as organoleptic observation, pH, viscosity, and density. Physical stability test performed are cycling test, storage in high temperature (40 ± 2°C), room temperature (25 ± 2°C), and low temperature (4 ± 2°C). In this accelerated stability test, stable hair tonics in 3 months storage stated as stable in 1 year [19].

Hair Growth Activity Test on Rats

Prior to the test, the rats had been acclimatized 2 weeks for adaptation to new environment. 24 rats were randomly divided into 6 treatment groups, each group contained 4 rats. Group I as normal control, group II as negative control applied only basis of hair tonic, group III was applied formula 1 (hair tonic with green tea leaves extract 2.5%), group IV was applied formula 2 (hair tonic with extract concentration 5.0%), group V was applied formula 3 (hair tonic with extract concentration 7.5%), and group VI as positive control, applied minoxidil 2.5% hair tonic.

a. Each of the rats was shaved 4 x 4 cm² on its dorsal skin, followed by applying depilatory cream to clean remaining hair on the area. For each test area, a square with area of 2 x 2 cm² was drawn. Then, the rats were left for 24 hours. Afterwards, samples were applied 2 ml once a day for consecutive 21 days on the test area. This test carried out for 3 parameters: Hair length determination

On day 7, 14, and 21 after treatments, 10 longest hairs were pulled randomly, each was measured, and then average length was calculated. The results are expressed as the mean length ± SD of 10 hairs [20][21].

b. Hair weight determination

On day 21, hair weight measurement was conducted by weighing whole hairs produced at the test area on day-21. Results expressed as hair weight ± SD from 4 rats in each treatment groups [20][21][22].

c. Hair diameter determination

Hair diameter was determined on day 21, to observe thickness of the hair, by using optical light microscope with magnification of 400x horizontally [21].

Statistical Analysis

Data obtained were analyzed using IBM software SPSS Statistic v.21. One way ANOVA test, followed by LSD (Least Significant Difference), was used for normal and homogenous data distribution. Whereas

for irregular and homogenous/not homogenous data distribution, nonparametric statistics were used i.e. Kruskal Wallis, followed by Mann-Whitney test with confidence level of 95%. Compared data with p<0.05 stated as significantly different, and p>0.05 stated as not significantly different [23].

Safety Test

Irritation test was conducted with 9 volunteers; all formulas were tested, each of the hair tonic formulas was applied to 3 volunteers. All tests concerning human were ethically approved by The Ethics Committee of the Faculty of Medicine, University of Indonesia, Indonesia, with regards of human rights and welfare in medical research. Volunteers had signed informed consent after comprehension of study protocol.

Following each test, upper arm skin of each volunteer was cleaned previously, and then 1ml of hair tonic was applied (2.5 cm x 2.5 cm). Each test area was covered with transparent plastic and wrapped in gauze pad for 24 hours, then unwrapped and washed, and observed if any redness, edema, or itch, subsequently. Observation was also performed after 48 hours. Obtained data was evaluated to conclude primary irritation index [24]:

$$PII = \frac{(\sum \text{erythema} + \sum \text{edema grade}) \text{ at 24 and 48 hr}}{\text{total volunteers} \times \text{number of observation}}$$

RESULTS AND DISCUSSIONS

Each component in this hair tonic formulation was determined in advance of preceding formula optimization, until it gave a fine quality of hair tonic. These components were ethanol 96%, propylene glycol, menthol, tween 80, sodium metabisulfite, methyl paraben, disodium edta, and aquadest. The composition of ethanol (96%) in these hair tonic formulations were using 75% of the formulation wherein lower amount of alcohol gave extract precipitations in room temperature.

The use of 75% ethanol (96%) in the formulation of this green tea hair tonic present advantages and disadvantages. Ethanol (96%) in topical dosage form is potential as penetration enhancer [25][26]. Grieco et al. (2010) [27] used minoxidil topical hair grower formulated in ethanol where it showed that formula with ethanol composition of 80% comparing to without ethanol gave significantly higher retention on stratum corneum through trans-appendageal route. Furthermore, research by Tata, Flynn, & Weiner (1995) [28] also stated penetration enhancement effect by ethanol on topical hair grower (using minoxidil as active ingredient), are essential. The research explained composition of ethanol (96%) enhance

penetration about 2 times higher with 50% of ethanol in the formulation, 3 times higher with 75% ethanol, and up to 8 times with 90% ethanol. This outcome resulted from the characteristic of ethanol that easily evaporate, bringing higher extract concentration on skin (increase of thermodynamic activity carried drug to pass stratum corneum), and/or because of ethanol changed physical integration of stratum corneum barrier, hence enhancing drug penetration. By the great chance of its absorption, regular use of ethanol showed relatively low concentration of alcohol and acetaldehyde metabolite in blood, lower from toxic concentration [29]. However, ethanol can present side effects such as skin irritation or dermatitis allergy although rarely reported. Other potential side effect is regarding high composition of ethanol, despite higher composition of ethanol increase penetration on skin, this would also be feasible for transdermal absorption of potential xenobiotic from carcinogenic contaminant from cosmetic formulation [29].

Other excipients used were propylene glycol, methyl paraben, sodium metabisulfite, and Na₂EDTA. Propylene glycol 10% was used as humectant and solubility enhancer in alcohol (cosolvent), furthermore 10% propylene glycol was known to prevent interaction between tween 80 and methyl paraben. Preservative used was only nipagin in small concentration (0.075%) without addition of propyl paraben, due to the use of ethanol in a high concentration. Ethanol has bactericidal function in mixture of ethanol-water 60-95% v/v, with optimum concentration of 70%. Moreover, the antimicrobial activity of methyl paraben was also enhanced by the presence of

Na₂EDTA. Along with its antimicrobial activity, methyl paraben also provides anti-fungal function. Other excipient, sodium metabisulfite, was used as antioxidant, preventing oxidation in GTE. In the other hand, Na₂EDTA was used as complexing agent, preventing oxidation caused by metals (catalyst of oxidation), and enhancing sodium metabisulfite's antioxidant activity [26].

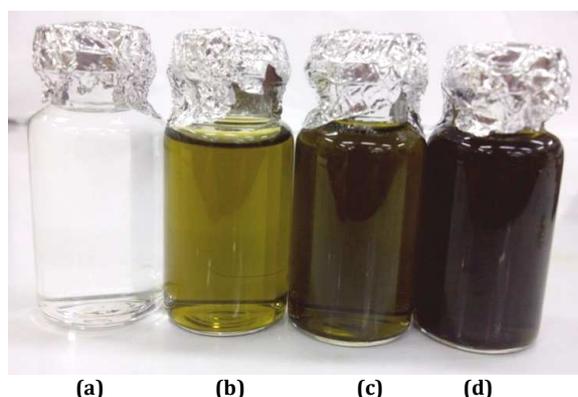
The results of the formulation of these GTE hair tonics were shown in Figure 1, these hair tonics were evaluated organoleptically. The most appealing hair tonic, aesthetically, was formula 1, with transparent yellowish green color (Pantone 583 C). Higher extract concentration darkened the color, given that the color of the GTE itself was dark green.

Further evaluations such as density, pH, and viscosity, were concluded in Table 2. The density of Formula 1, 2, and 3 were 0.8961341, 0.8839836, 0.8918725 gr/ml, respectively. The density of these hair tonics was lower than water density, due to high composition of ethanol. The pH of the hair tonics, Formula 1, 2, and 3 initially was 5.57, 5.34, and 5.23, respectively. This showed higher concentration of the extract lower the pH of the hair tonic, because the presence of phenolic acid contained in the GTE. The range of pH of these hair tonics was suitable for pH skin (pH 4.5 - 6.5). Alkali hair tonics would cause dryness to skin, whilst acidic hair tonics would cause itchiness. As shown in Table 2, viscosities of these hair tonics were not different significantly, where increase of viscosity were because of the increase of the concentration of GTE.

Table 2: It shows initial evaluation results from each formulation of hair tonics

	Color	Opacity	Odor	Homogeneity	pH	Density (gr/ml)	Viscosity (mPa.s)
F1	Yellowish Green+	Transparent	Specific scent	Homogen	5.57	0.8961	0.2280
F2	Brownish Green++	Transparent	Specific scent	Homogen	5.43	0.8840	0.2350
F3	Dark Green+++	Transparent	Specific scent	Homogen	5.23	0.8920	0.2371

Where, F1 = Formula 1 (green tea ethanolic extract 2.5%), F2 = Formula 2 (green tea ethanolic extract 5.0%), F3 = Formula 3 (green tea ethanolic extract 7.5%), Yellowish Green+ = Pantone 583 C, Brownish Green++ = Pantone 581 C, Dark Green+++ = Pantone 5747 C



Where, (a) Negative Control, (b) Formula 2.5% (c) Formula 5.0%, (d) Formula 7.5%

Fig. 1: It shows formulated GTE in hair tonics

Evaluations were continued to physical stability test by conducting cycling test and storage in varied temperatures with observing parameter of organoleptic, pH, and viscosity. Cycling test was performed to monitor stability of the hair tonic in changing temperature, where the result of this test showed all of the hair tonics were stable. The hair tonics were still homogenous, although its opacity increased. In the other hand, storage in room temperature (25°C ± 2°C) and high temperature (40°C ± 2°C) showed that hair tonics were stable organoleptically. However, in low temperature (4°C ± 2°C), precipitation was formed on all formulas. This showed lowering temperature decreased solubility of the extract, though if moved to room temperature, it became homogenous again (no precipitation). Observation of pH profile showed variability yet still acceptable. Figure 2 showed inconsistency of pH,

however the change of pH was still in the range of skin acidity (pH 4.5-6.5), and the range of green tea catechin stability (pH 5 or below pH 4) [30][31][32]. As for viscosity, storage results after 12 weeks showed stability in spite of its not significant slight increase. Generally physical stability test of these hair tonics showed stability, however, storage of this GTE hair tonic was not recommended in low temperature. Based on physical evaluation study and physical stability test, formula 1 (GTE concentration 2.5%) was the best formula. All of the formulas gave stability in physical stability test on storage in room temperature (25°C ± 2°C), and high temperature (40°C ± 2°C), except in low temperature (4°C ± 2°C) wherein it showed to be not homogenous (precipitation formed). Cycling test gave stable results in all of the formulas. Aesthetically, formula 1 showed to be the most appealing hair tonic.

Following the stability test, significant results rested in activity towards hair growth test using *Sprague Dawley* rats. Analysis conducted were comparison between activity of normal and negative control, negative control and positive control (i.e. minoxidil 2.5%), positive control and green tea hair tonics nutraceutical formulas, and comparison between all hair tonic formulas (i.e. 2.5%, 5.0%, and 7.5% GTE concentration). The test performed in 21 days with depicted visual observation (Figure 3), measurements of hair length day-7, 14, and 21 (Table 3), weights of total hair mass on day-21 (Table 3), and measurements of diameter (Table 4).

Data comparison between normal control and negative control on day-7, 14, and 21 showed to be not significantly different ($p > 0.05$). For hair weight data, according to Mann-Whitney statistical analysis, presented no significant difference ($p > 0.05$). Diameter measurements on point 1 (lower part, 0-9 mm from hair follicle) and point 3 (upper part, 18-27 mm from hair follicle) also showed no significant difference, although on the point 2 (middle part, 9-18 mm from hair follicle) the data supported deviant results as significantly different where normal control ($9.69 \pm 0.93 \mu\text{m}$) were thicker

comparing negative control ($7.19 \pm 0.16 \mu\text{m}$). The justification currently could not be concluded, though it is known that the composition of alcohol in the hair tonics was high,

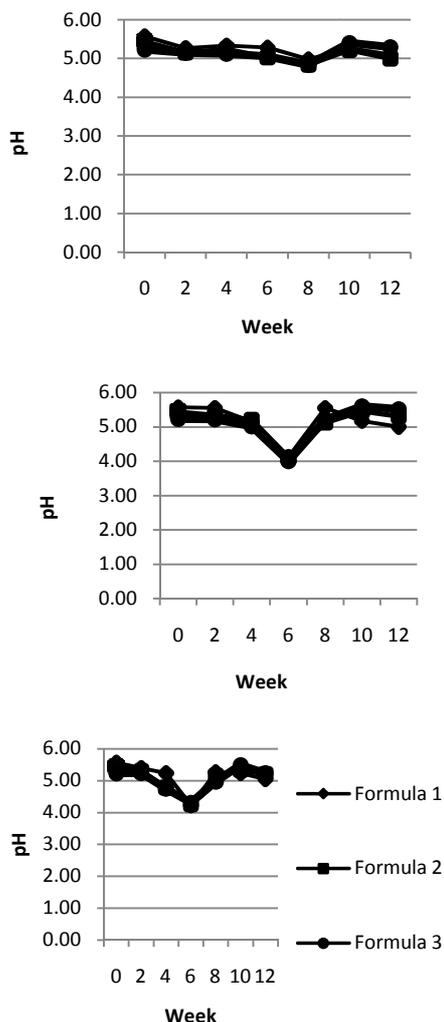


Fig. 2: It shows pH profile in 12 weeks storage in low temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$), room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), and high temperature ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$)

Where, (a) storage in low temperature ($4^{\circ}\text{C} \pm 2^{\circ}\text{C}$), (b) room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) (c) high temperature ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$)

whether this was proccurring cause or not, further research is needed. Generally, the results demonstrated that hair growth activity of normal control were equal to negative control, thus it was concluded that the control negative did not enhance hair growth activity.

Comparison between positive control and negative control generally on whole observation day can be observed in Table 2. Statistically, these data clarified that mean hair length of positive control were higher comparing to negative control in which resulted significant different ($p < 0.05$). On the contrary, weight measurements showed no significant difference ($p = 0.558$), along with diameter measurements presenting no significant difference ($p = 0.929$). This case might be due to total hair count per area of positive control was less than negative control. Nevertheless, main conclusion of positive control's hair growth activity comparing to negative control was still superior because of the means of hair length of positive control was longer than negative control ($p < 0.05$).

Between GTE formulas and positive control on day-7, 14, and 21 generally showed significant different in mean hair length and weight on day-21 statistically ($p < 0.05$). In Table 2, it was expressed that the means of hair length of F1, F2, and F3 from each observation were longer than positive control minoxidil 2.5%. Mann-Whitney statistical analyses showed significant difference ($p < 0.05$) considerably, except on day-21, F1 did not exhibit significant difference to positive control. Further data of weight measurements on day-21 Formula 1, 2, and 3 i.e. 22.20 mg/cm^2 , 19.93 mg/cm^2 , 15.39 mg/cm^2 , respectively, comparing to the weight of positive control, 10.91 mg/cm^2 , again showed significant difference ($p < 0.05$) with LSD. Despite, diameter comparison between F1, F2, F3 and positive control on day-21 demonstrated no significant difference ($p > 0.05$). After all of aforementioned data, it was concluded that green tea ethanolic extract hair tonic 2.5%, 5.0%, and 7.5% had greater activity than minoxidil 2.5% generally.

The comparison of hair length and total weight between formula 1, formula 2, and formula 3 in all observations did not principally present significant different. The means of rats' hair length on day 7 and day 14 were not significantly different ($p > 0.05$). On day 21, formula 2 gave higher means of hair length, yet formula 1 gave higher total hair weight although not significantly higher ($p = 0.422$). On the other hand, hair diameter on day 21 among all formulas showed no significant difference ($p > 0.05$).

These data presented that even in lower concentration i.e. F1 with GTE 2.5%, indicate equivalent hair growth promoting activity comparing to higher concentration (F2, 5.0%; F3, 7.5%). In conclusion, the most optimum hair tonic was formula 1, containing green tea ethanolic extract 2.5%.

Table 3: It shows mean of hair length results from each treatment on day-7, 14, 21 and mean of hair weight determination results on day 21

Treatments Group	Treatments	Means of hair length (mm) \pm SD			Mean of Hair Weight (mg/cm^2) \pm SD
		Day 7	Day 14	Day 21	
I	Normal Control	1.95 ± 1.95	10.30 ± 1.57	14.28 ± 1.08	11.73 ± 0.66
II	Negative Control	6.89 ± 0.08	8.27 ± 0.48	14.00 ± 1.36	9.26 ± 2.70
III	Formula 2.5%	14.69 ± 1.24	22.34 ± 0.59	23.75 ± 1.22	22.20 ± 0.27
IV	Formula 5.0%	16.98 ± 1.70	22.53 ± 0.83	24.64 ± 0.70	19.93 ± 2.61
V	Formula 7.5%	16.47 ± 0.46	22.33 ± 0.36	24.22 ± 0.84	15.39 ± 2.59
VI	Positive Control 2.5%	10.99 ± 0.42	18.44 ± 1.25	21.02 ± 1.22	10.91 ± 1.26

Table 4: It shows the mean results of hair diameter measurements on day 21

Treatments	Hair Diameter (μm) \pm SD		
	Point 1	Point 2	Point 3
Normal Control	6.63 ± 1.14	9.69 ± 0.93	4.19 ± 0.12
Negative Control	6.13 ± 0.39	7.19 ± 0.16	4.38 ± 0.39
Formula 1 (2.5%)	5.31 ± 0.19	7.25 ± 0.10	4.38 ± 0.54
Formula 2 (5.0%)	4.75 ± 0.44	8.56 ± 0.43	4.63 ± 0.16
Formula 3 (7.5%)	5.13 ± 0.36	7.88 ± 0.58	4.63 ± 0.39
Positive Control	5.38 ± 0.38	7.25 ± 0.18	4.88 ± 0.88

Where, point 1 = 0 - 9 mm from hair follicle, point 2 = 9 - 18 mm, point 3 = 18 - 27 mm.



Where, (a) Normal Control, (b) Negative Control, (c) Formula 2.5%, (d) Formula 5.0%, (e) Formula 7.5%, (f) Positive Control (minoxidil 2.5%)

Fig. 3: It shows visual observation results of hair growth on day 7, 14, and 21

Safety test was performed subsequent to hair growth activity test. Results showed no erythema, eschar, or edema after 24 hours and 48 hours on Formula 1, Formula 2, and Formula 3. Since the PII score (Primary Irritation Index) is 0, so the category for respond and irritation is affirmed as no irritation (0-0.4). Hence, all of the hair tonics are safe to use.

CONCLUSION

Based on the results from physical stability test, hair growth activity test, and safety test from GTE hair tonics in varied concentration i.e. 2.5%, 5.0%, and 7.5%, it is concluded that;

- a. Hair tonic containing GTE 2.5%, 5.0%, and 7.5% showed physical stability at room temperature storage, (25°C ± 2°C), and at high temperature (40°C ± 2°C) in 12 weeks. In exception of storage at low temperature (4°C ± 2°C), hair tonic showed unhomogenous, so it's suggested not to store in low temperature.
- b. All of the formula showed activity towards hair growth. Moreover it showed greater activity than minoxidil 2.5%, whereas formula 1 (2.5%) gives the best activity.
- c. All of the formula are safe to use and did not irritate skin.

To conclude, GTE has demonstrated such potential hair growth activity which is comparable to minoxidil as synthetic drug. Researches concerning GTE shall be continued, especially in achieving more stable and safer dosage form, further research on formulation of GTE using current technology would be worth to develop.

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CONFLICT OF INTEREST

This research has no conflict of interest.

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