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Formulation and Evaluation of Water Fraction Hair Tonic Containing Flavonoids from Ethanolic Extract of Green Tea Leaves (*Camellia sinensis* L.)

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ABSTRACT

Hair growth tonics containing herbal and synthetic ingredients have been developed to overcome hair loss and baldness. Advanced technological developments made many Indonesians prefer to use herbal products compared to synthetic products due to their fewer side effects. Green tea leaves (*Camellia sinensis* L.) is a plant believed to increase hair growth rates due to its flavonoid compounds. The purpose of this study is to formulate hair tonic from water fraction ethanolic extract green tea leaves, to look at the activity of hair growth-promoting, and also to look at physical stability, irritation tests, and microbial contamination. The positive control used is 2% Minoxidil. The hair growth activity test was carried out by applying a hair tonic to rabbits. Hair growth measurement data were statistically tested by the ANOVA test. The formulated green tea leaves tonic met the physical properties test. The tonic produces similar growth activities with the positive control (significance difference ($p > 0.05$)). In addition, the tonic does not have a skin irritation effect on rabbit skin and is free from bacteria.
Keywords: Green tea leaves; hair growth activity; water fraction; ethanolic extract; hair tonic formula.

INTRODUCTION

Hair has an important role in humans. Hair regulates temperature, facilitates sweat evaporation, and stimulates a sensitive sense of touch. Hair loss can be caused by several factors, including age, genetics, certain races hereditary, hormonal, immunological, nutritional deficiencies, psychological stress, physical trauma, skin diseases, systemic diseases, systemic drugs, and other unknown causes.

Hair loss is a natural phase that occurs in everyone. The growth phase in the hair includes the anagen phase (growth), the catagen phase (to fall out), and the telogen phase (fall out) (Amin and Sakhdeva, 2013). On average, people lose 50-100 strands of hair every day due to fallout. Normally all hair loss is replaced with new growth of new hair. However, a continual hair loss of more than 100 strands per day is a feature of unhealthy hair.

To prevent hair loss, a haircare procedure needs to be performed. Haircare involves the use of a variety of cosmetics, ranging from hair cleaning cosmetics, hair conditioners, cream baths, and also hair tonics. An easy way to treat hair loss is by using a hair tonic as an ingredient to nourish hair. Hair tonic contains ingredients that are needed by the hair, hair roots, and scalp.

Various hair care products from both synthetic and natural ingredients have been developed to overcome hair loss problems. One of them is available on the market prepared from synthetic substances such as Minoxidil. Minoxidil stimulated secondary hair germ cells of telogen follicles and caused a rapid shift to the anagen phase and also stimulated prostaglandin E_2 production (Suchonwanit *et al*., 2019). But, Minoxidil has potential side effects such as skin allergies, headaches, vertigo, edema to hypotension (Messenger and Rundegren, 2004). Alternatively, many herbal plants in Indonesia can be used as ingredients for hair growth treatment such as green tea leaves (*Camellia sinensis* L.) which contain flavonoid compounds (Bassino *et al*., 2020).

In the field of cosmetics, green tea can be used as anti-aging and anti-wrinkle, photo-protection, anti-acne, whitening, stretchmarks, anti-microbial, anti-dandruff, hair grower (Kumar *et al*., 2012). Flavonoid in green tea are epigallocatechin-3-gallic (EGCG). EGCG has been reported to increase the proliferation of dermal papilla cells (DPC) and outer root sheath cells (Zhang et al., 2018). EGCG promotes hair growth by activating Erk and Akt signaling and increasing the B-cell lymphoma 2/Bax ratio in DPC (Shin et al., 2016). The benefits of green tea leaves as a hair growth-promoting have been proven by Lamria (2013) that 2.5% green tea leaves extract in hair tonic preparations increased hair growth rates significantly compared to Minoxidil 2.5%. Amin *et al.,* (2014) proved that ethanolic green tea

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Table I. Hair tonic preparation basic formula

Material	Concentration (%)
Water Fraction	4
96% Ethanol	30
Propylene glycol	10
Phenoxyethanol	0,8
Distilled Water	add 100

extract in varied concentrations i.e. 2.5%, 5%, and 7.5% giving hair growth activity, all of the concentrations had greater activity than synthetic drug minoxidil 2.5%. Noviani *et al.* (2019) also proved that the ethyl acetate and water fractions of ethanolic extract of green tea leaves contained flavonoids, so it could continue the hair growth-promoting activities with concentrations of 1% and 4%, but the result showed that 4% of water fraction containing flavonoids had the best hair growth-promoting activity.

Based on the data above, in this study, 4 % of the water fraction of ethanolic extract green tea leaves will be formulated to be hair tonic. This study aims to formulate hair tonic from water fraction ethanolic extract green tea leaves, look activity of hair growth-promoting, and also to look physical stability, irritation tests, and microbial contamination. Minoxidil was used as a positive control in this study because the reason for this study was to find natural compounds that can replace synthetic compounds that have side effects.

METHODOLOGY

Materials

The materials used are green tea leaves, 70% ethanol, 2% minoxidil, 96% ethanol, propylene glycol, phenoxyethanol, and distilled water.

Methods

Determination of Green Tea Leaves

Plant determination was carried out at the Herbarium Bogoriense, Botanical Sector, Center Biological Research-LIPI, Cibinong.

Preparation of Ethanolic Extract and Water Fraction

Ethanolic extract from green tea leaves and water fraction was made refers to the method of Noviani *et al.* (2019). The simplicia from green tea leaves used was 2000 grams. One part of dry powder was added 5 parts of 70% ethanol. The powder is soaked in 70% ethanol for one day and stirred every 6 hours. The obtained macerate was separated by filtration using a flannel cloth. This process was repeated for five days using the residue of simplicia powder. All the macerates were collected. The macerate was concentrated with a vacuum evaporator at the temperature of 50^0C and heating it over a water bath until the solvent evaporated perfectly or viscous extract is obtained. The viscous extract was fractionated using the liquid-liquid partition method in the separating funnel with n-hexane: water (1:1) to obtain n-hexane and water fractions separately. The water fraction obtained then fractionated using ethyl acetate: water (1:1) to obtain ethyl acetate and water fraction. The water fraction obtained is dried using the freeze-drying method.

Preparation of Hair Tonic

The formula for hair tonic can be seen in Table 1. The water fraction of green tea leaves was dissolved with some distilled water and then ethanol was added and stirred until a homogeneous state. Afterward, propylene glycol was added and stirred for a homogeneous state. Lastly, phenoxyethanol and the rest of distilled water were added to the mixture. The positive control used is 2% Minoxidil.

Hair Tonic Evaluation

Hair tonic evaluation includes organoleptic, pH, and viscosity. The organoleptic test included visual observation on color, smell, clarity, and homogeneity. The pH was measured with a pH meter that had been calibrated by dipping the electrode in two solutions under the assumption that the pH of the test solution was between the pH of the two solutions. The commonly used solutions are pH 4 and pH 7. The viscosity was identified using the Ostwald viscometer by measuring the time that the liquid was required to pass through two marks as it flowed through a vertical capillary tube.

The Procedure for Animals

The animal used were 4 male white rabbits aged 3-4 months with an average weight of 1.8 to 2.5 kg. Before the experiment, rabbits need to be

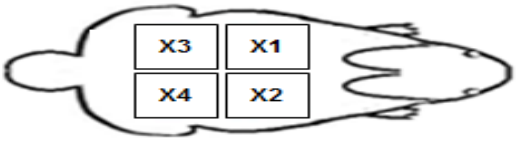


Figure 1. The position of the treatment area

X1: positive control (2% minoxidil); X2: negative control (base); X3: smeared with hair tonic formula and X4: normal control (not smeared)

adapted in advance to the place, cage, and food for one week. During the adaptation and the test, the animals were given the same type and amount of water and nutrition.

The Procedure of Hair Tonic Activity Test

This study is referred to a method from Tanaka *et al.,* (1980). Each rabbit received 4 different treatments. The rabbit's back is shaved by making 4 areas with 2.5 x 2.5 cm rectangular shapes with a 1 cm distance between shapes. Before tonic application, the rabbit's back was treated with 70% ethanol as an antiseptic. The application area is as follows Figure 1.

The test solution was administered topically on the rabbit shaved skin, twice a day (morning and evening) with a volume of 1 mL, for 28 days. On days 7, 14, 21, and 28 from each treated area was taken randomly 6 hairs and the length of each hair was measured. Hair is taken by cutting, then straightened and placed on a dark base as well as tape and measured using a caliper. On the 28th day, all hair in each treated area is cut and weighed.

Procedure for Microbiological Test

The total plate number (TPN) was performed in this study. This study used the pouring method and was left for 24-48 hours at 35-37°C. The number of colonies that could grow on plates was counted manually with "Colony Counter". The number of colonies that met the calculation requirements was 30 to 300 colonies on plate media.

Procedure for Irritation Test

An acute irritation test was performed on a healthy adult rabbit. The test was carried out three times. Rabbits were shaved in the back area of approximately 10x15 cm. The dosage used for the liquid test preparation was 0.5 ml. The preparation was applied first to a gauze pad and then placed on the skin. The test preparation was exposed to an area of ± 6 cm^2 in the skin then the exposure location was covered with a gauze pad and an adhesive bandage for 4 hours.

All test animals were observed for the presence of erythema and edema. Response assessment was carried out at 1, 24, 48, and 72 hours after opening the patch. If skin damage, as irritation or corrosion, is not observable at 72 hours, the observation is continued until day 14 to determine the reversibility (BPOM, 2014)

Data Analysis

Kruskal-Wallis and Mann-Whitney (ANOVA) were used to determine the statistical significance ($p < 0.05$) of the differences between the values of various group.

RESULT AND DISCUSSION

In this study, hair tonic preparations were made from the 4% water fraction of ethanolic extract green tea leaves. Hair tonic preparations are selected due to their advantages in terms of ease of manufacture and use without stickiness in their appliance. In the formulation, 96% ethanol is used as a co-solvent and enhancer. Propylene glycol is used as a humectant and phenoxyethanol is used as a preservative. The hair tonic that was made in this study can be seen in Figure 2.

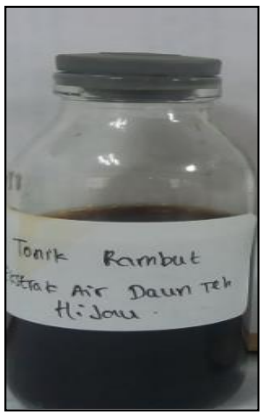


Figure 2. Hair Tonic Preparations

Table II. Stability Test Result

Day	Temp (°C)	Organoleptic				pH	Viscosity (cps)
		Color	Odor	Clarity	Homogeneity		
0	4	Brownish red	Odorless	Clear	Homogenous	5.5	10.5
	25	Brownish red	Odorless	Clear	Homogenous	5.5	10.5
	40	Brownish red	Odorless	Clear	Homogenous	5.5	10.5
15	4	Brownish red	Odorless	Clear	Homogenous	5.5	11.2
	25	Brownish red	Odorless	Clear	Homogenous	5.5	10.8
	40	Brownish red	Odorless	Clear	Homogenous	5.5	10.8
30	4	Brownish red	Odorless	Clear	Homogenous	5.5	12.0
	25	Brownish red	Odorless	Clear	Homogenous	5.5	11.2
	40	Brownish red	Odorless	Clear	Homogenous	5.5	9.6
40	4	Brownish red	Odorless	Clear	Homogenous	5.5	10.3
	25	Brownish red	Odorless	Clear	Homogenous	5.5	10.6
	40	Brownish red	Odorless	Clear	Homogenous	5.5	10.4

Table III. Weekly average hair growth results

Treatment	Average length (mm)			
	Week			
	1	2	3	4
Normal control	1.23	2.80	4.83	6.32
Base	1.48	3.30	5.63	7.39
Positive Control	4.97	8.91	10.61	14.66
Hair tonic	5.26	8.59	10.03	14.89

The hair tonic was evaluated for physical stability. The result of the physical stability test of the hair tonic is summarized in Table II. The organoleptic results do not show any changes in odor, homogeneity, and clarity. Therefore, the hair tonic preparation was stable. The hair tonic has a pH of 5.5 still in the range of pH balance of skin (4.5-7.5) which is considered safe to use for the skin. The result of viscosity in three temperatures produces different viscosity but is close to the initial viscosity. The possibility of a change in viscosity to be higher or lower is due to the measurement of the newly released preparation from the oven has not reached room temperature. Therefore, the viscosity value tends to be higher or lower.

The test of hair growth activity is performed by observing the average hair length and weight of rabbit hair. The average rabbit hair length's result can be seen in Table III and Figure 3. The all treatment group show an increase in the average hair length compared to the normal control. The greatest increase in hair length occurred in the hair tonic and positive control groups.

The results of the weight of rabbit hair per week can be seen in Figure 4 and the average hair weight can be seen in Table IV. Hair tonic groups and positive controls have a better hair thickness than the normal and base groups. Negative control and the base hair have a lower weight compared to the hair tonic and positive control.

Statistical analysis for the length and weight of rabbit hair showed that the data were distributed abnormally and not homogeneously. The *Kruskal Wallis* test showed significant differences between treatment groups ($p < 0.05$). It means that each treatment produces a different hair growth performance. The *Mann-Whitney* test showed a significant difference between all groups ($p < 0.05$). The positive control with the hair tonic group does not produce a significant difference ($p > 0.05$). It means that hair tonic preparations and positive controls have the same performance as hair growth-promoting.

Green tea leaves contain secondary metabolites which play a role in stimulating hair growth. Flavonoid compounds as one of the many phenolic compounds in plant tissues can act as antioxidants. Free radicals are one of the causes of hair loss. Flavonoids can prevent free radicals and accelerate hair growth. In addition, the hair growth mechanism of epigallocatechin-3-gallate (EGCG)

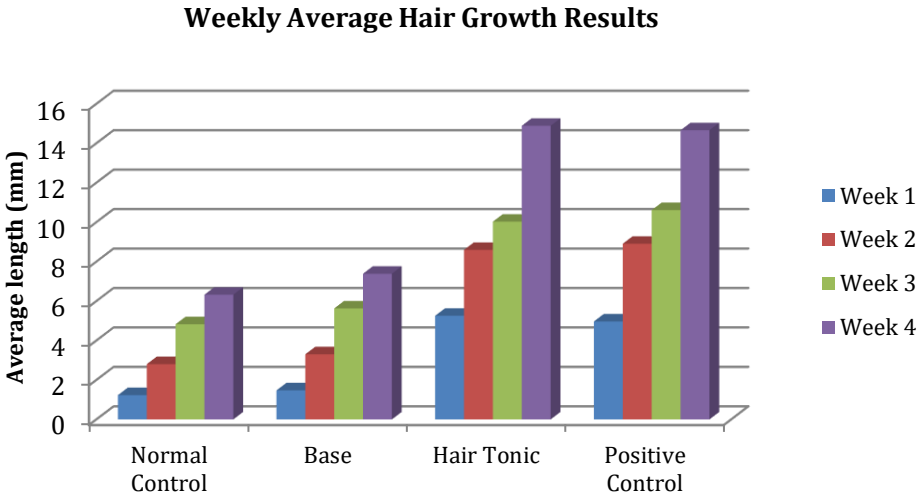
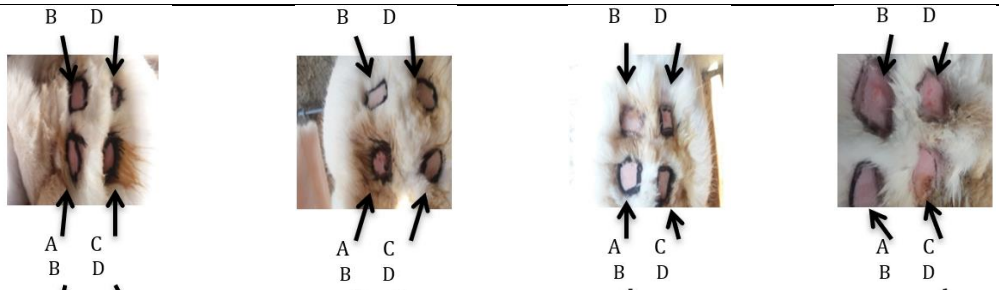
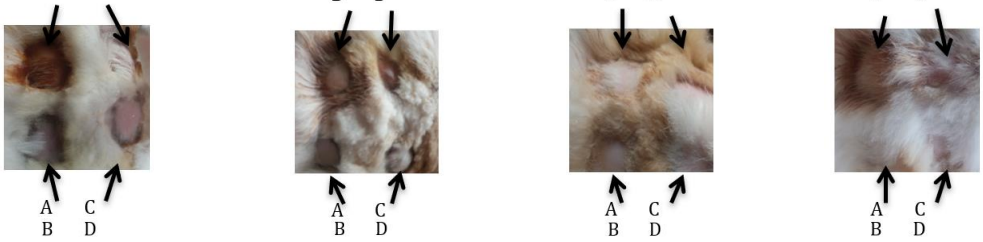
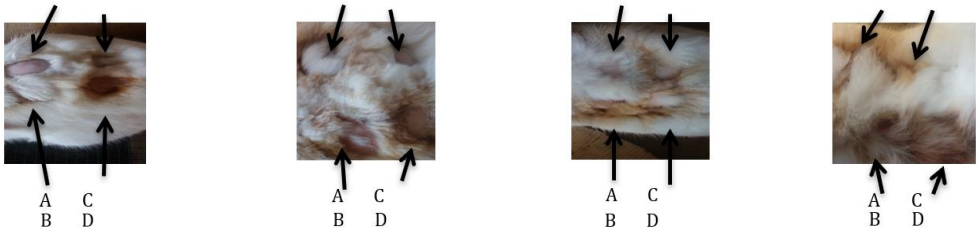
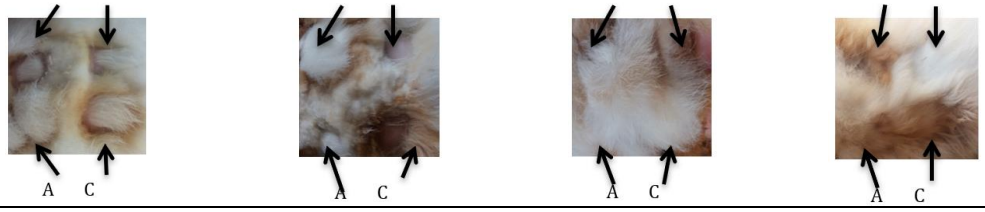


Figure 3. Weekly Average Hair Growth Results

Week	Rabbit			
	1	2	3	4
1				
2				
3				
4				

A = Positive control; B = Base; C = Hair Tonic; D = Normal Control

Figure 4. Hair Growth of Rabbit During 4 Weeks

Table IV. Average Hair Weight on Day-28

Treatment	Average (mg) ±SD
Normal Control	38.00 ± 4.27
Base	74.33 ± 6,70
Positive Control	209.67 ± 6.70
Hair tonic	171.67 ± 25.57

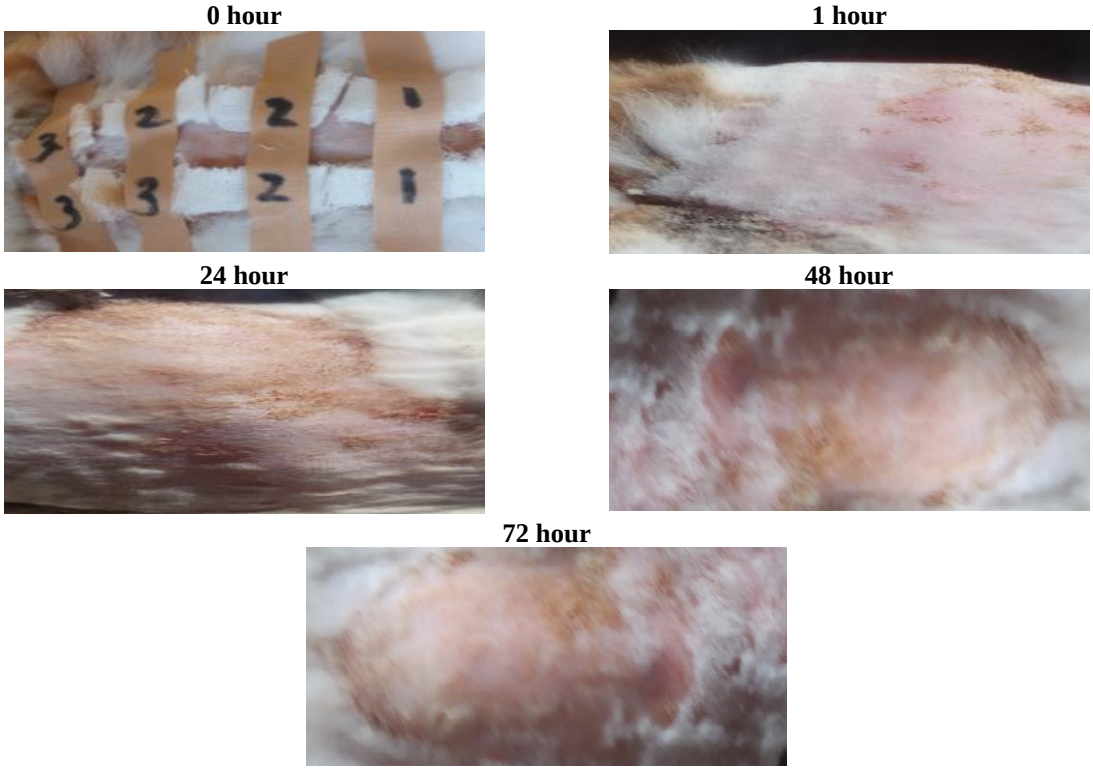


Figure 5. The Irritation Test Result

by activating Erk and Akt signaling and increasing the B-cell lymphoma 2/Bax ratio in DPC (Shin *et al.,* 2016).

Hair tonic preparations contain a large number of organic solvents. The use of many organic solvents can irritate the skin, so an irritation test should be carried out to see the hair tonic is safe or irritating. The irritation test on hair tonic showed that green tea leaves do not irritate the rabbit skin. The previous research about the toxicity of green tea leaves extracts also proved that green tea extract did not show any sign of irritation in the skin (Kim *et al*., 2012). The figure for this test can be seen in Figure 5.

The microbiological test of hair tonic showed that the total number of bacteria for hair tonic preparations is 0 colonies per ml, which means that the results meet the requirements of microbial contamination in cosmetics.

CONCLUSION

The hair tonic of water fraction from ethanolic extract green tea leaves was stable physically. Hair tonic produces hair growth activities similar to the positive control, does not irritate the rabbit skin, and is free from microbial contamination.

REFERENCES

Amin J., Simamora ELP., Anwar E., Djajadisastra J., 2014, Green Tea (Camellia sinensis, L.) Ethanolic Extract As Hair Tonic In Nutraceutical: Physical Stability, Hair Growth Activity On Rats, And Safety Test, *International Journal of Pharmacy and Pharmaceutical Sciences,* 6 (5), 94-99.

Amin SS., Sachdeva S., 2013, Alopecia areata: A review, *Journal of the Saudi Society of Dermatology & Dermatologic Surgery*, 17

(2), 37-45.

Bassino E., Gasparri F., Munaron L., 2020, Protective Role of Nutritional Plants Containing Flavonoids in Hair Follicle Disruption: A Review, *Int. J. Mol. Sci*, 21 (523), 1-17.

BPOM, 2014, *Peraturan Kepala Badan Pengawasan Obat dan Makanan Republik Indonesia nomor 7 tahun 2014 tentang Pedoman Uji Toksisitas Nonklinik secara In Vivo*, Badan Pengawasan Obat dan Makanan Republik Indonesia, Jakarta, pp. 55.

Kim HK., Choi SY., Chang HK., *et al,* 2012, Human skin safety test of green tea cell extracts in condition of allergic contact dermatitis, *Toxicol Res*, 28 (2), 113-116.

Kumar S., Singh N., Shweta, Archana, 2012, Green Tea Polyphenols: Versatile Cosmetic Ingredient, *IJARPB*, 2 (3), 348-362.

Lamria E., 2013, Uji Stabilitas Fisik, Aktivitas, Dan Keamanan Dari Sediaan Hair Tonic Yang Mengandung Ekstrak Etanol Teh Hijau (Camellia sinensis L) sebagai Nutrasetika, *Skripsi*, Fakultas Farmasi Universitas, Jakarta.

Messenger AG., Rundegren J., 2004, Minoxidil: Mechanism of Action on Hair Growth, *British Journal of Dermatology*, 150 (2), 186-194.

Noviani, V., Thauresia S., Apriani, E.F., & Simanjuntak, P., 2019, Hair Growth Promoting Activity of Green Tea Leaves (Camellia sinensis l.) Ethanolic Extract, *Trad. Med. J*., 24 (2), 104-109.

Shin S., Kim K., Lee MJ., *et al.,* 2016, Epigallocatechin Gallate-Mediated Alteration of the MicroRNA Expression Profile in 5α-Dihydrotestosterone-Treated Human Dermal Papilla Cells, *Ann Dermatol*, 28 (3), 327-334.

Suchonwanit P., Thammarucha S., Leerunyakul K., 2019, Minoxidil and its use in hair disorders: a review, *Drug Des Devel Ther*, 13, 2777-2786.

Zhang H., Nan W., Wang S., *et al,* 2018, Epigallocatechin-3-Gallate Promotes the Growth of Mink Hair Follicles Through Sonic Hedgehog and Protein Kinase B Signaling Pathways, *Front Pharmacol*, 9 (674), 1-11.