



Hair Growth Activity Test of Hair Tonic That Contain Green Tea Leaf Extract, Celery Leaf Extract and Combination of Green Tea Leaf and Celery Leaf Extract

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ABSTRACT

*Hair loss is a abnormality where the amount of hair is less or more than normal with or without visible thinning. Excessive hair loss can lead to baldness. The prevalence of hair loss in women aged 14-28 years in Jakarta is 50%, and in Surabaya it occurs in 53.3% of hijab users. One of the formulations that could be used to prevent hair loss is hair tonic formulations. Hair tonic is a cosmetic formulation that functions to stimulate hair growths, both in normal hair and in hair loss and baldness. Hair tonic can be developed from natural ingredients such as green tea leaves, celery leaves or a combination of both. Green tea leaf contains epigallocatechin-3-gallate and flavonoids which have hair growth activity, while celery leaf (*Apium graveolens L.*) contains apigenin compounds which have hair growth activity. This research aims to formulate and evaluate hair tonic containing ethanol extract of green tea leaves (*Camelliasinensis L.*), celery leaf (*Apium graveolens L.*) and combination of ethanol extract of green tea leaves (*Camelliasinensis L.*) and celery leaf (*Apium graveolens L.*) in guinea pigs. This study is an experimental type of quantitative research with a true experimental design, laboratory and Completely Randomized Design (CRD). Hair tonic formulations were made with 6 formulations given to 6 groups of guinea pigs with a total of 4 guinea pigs in each group. The formulations used were negative control, positive control of celery leaf extract, positive control of green tea leaf extract, formulation of 1 green tea leaf 7.5% and celery leaf extract 2.5%, formulation of 2 green tea leaf 5% and celery leaf extract 5%, formulation 3 green tea leaf extract 2.5% and celery leaf extract 7.5%. Evaluation of hair tonic formulations was carried out by physical evaluation with organoleptic tests and pH tests. Evaluation of hair growth activity observed the hair growth of guinea pigs every 7 days for 28 days and measured it with a caliper. The results of the organoleptic test of organoleptically stable hair tonic formulations were assessed from the color, smell, and texture that did not change. The results of the pH test met the requirements for the skin's pH value of 4.5-6.5. The results of the hair growth test on hair tonic formulations showed that all formulations had the ability to grow hair in guinea pigs. Green tea leaves (*Camellia sinensis L.*) extract, celery leaves (*Apium graveolens L.*), and the combination of green tea leaves (*Camellia sinensis L.*) extract and celery leaves (*Apium graveolens L.*) extract can be formulated and used as hair growth in guinea pigs. The best grow hair fast formulation is combination of green tea leaves extract : celery leaves extract is 2.5%:7.5%.*

KEYWORDS: hair growth, hair tonic, combination of green tea and celery leaf.

INTRODUCTION

Hair has a very important benefit to the environment, namely protecting the scalp from heat, cold temperatures and ultraviolet rays (Choi, 2018; Signori, 2004). Hair growth generally has 3 main phases, namely anagen, catagen and telogen (PK & Dass, 2015). Hair

loss is a difference where more hair is released than normal. Normally, 80-120 strands of hair are shed per day (Rassman & Bernstein, 2008). The incidence of hair loss is still quite high, in the United States hair loss has afflicted 50 million people and 20 million of them are women, in Jakarta the prevalence of hair loss in women aged 14-28

years is 50%, and in Surabaya the incidence of hair loss is 53,3% of hijab users at the Faculty of Medicine, Wijaya Kusuma University. Hair loss occurs in more than 90% of men and about 30% of women during their lifetime (Jafar et al., 2017; Wahyuni et al., 2020). Hair loss can be caused by hormones, genetic disorders, systemic diseases, nutritional status, intoxication, environmental stimuli, and hair cosmetics (Lase, 2019). Excessive hair loss can lead to baldness (Mounsey & Reed, 2009). There are only two baldness drugs approved by the Food and Drug Administration (FDA) or the United States Food and Drug Administration, so that the development of herbs for the prevention and treatment of baldness is one of the alternatives developed (Albaihaqi & Mustarichie, 2019). Formulations that can be used to treat hair loss are hair tonic formulations. Hair tonic is a cosmetic formulation that functions to nourish hair, both for hair loss, normal hair and baldness (Mulyanti et al., 2019).

Hair tonic can be developed from natural ingredients such as celery leaves, green tea leaves or a combination of both. Green tea leaves (*Camellia sinensis* L.) have long been consumed as a safe food, and have no adverse side effects if consumed for a long time. Traditionally green tea leaves have many benefits that can be used to treat gastrointestinal infections, cholesterol and high blood pressure, diabetes, headaches, diarrhea, hair growth and hair blackening.

Hair tonic formulations from ethanol extract of green tea leaves have hair growth activity. Green tea leaves contain pigalocatechin-3-gallate and flavonoids which have hair growth activity (Noviani et al., 2019). Celery leaf (*Apium graveolens* L.) is a plant that is widely used in daily life as a complementary food and as a medicine for rheumatism, high blood pressure, high cholesterol and stimulates hair growth. Celery leaf extract with a concentration of 10% in hair tonic formulations turned out to have an optimal hair growth effect (Jubaidah et al., 2018). Celery leaves contain apigenin compounds which have activity as hair growth (Kuncari et al., 2015). This research aims to determine the hair growth activity of the combination of ethanol extract of green tea leaves (*Camellia sinensis* L.) and celery leaves (*Apium graveolens* L.) in the form of hair tonic formulations in guinea pigs.

MATERIAL AND METHODS

Chemicals and Instrument

The tools used include analytical scales, watch glass, stir bar, spray bottle, dropper, rotary evaporator B-One RE-2010, beaker glass, universal pH meter, funnel, jar, shaver, marker, tweezers, caliper. The ingredients used include celery leaf extract, green tea leaf extract, menthol, sodium metabisulfite, tween 80, 96% ethanol, propylene glycol, methyl paraben, propyl paraben, Na₂EDTA and distilled water.

This research was conducted at the Pharmacy Laboratory of the Faculty of Health Sciences, Nahdlatul Ulama University Sunan Giri Bojonegoro for 3 months starting from April to July 2021. An experimental research with quantitative research type with a true experimental laboratory design and Completely Randomized Design (CRD). The research population was the hair of 30 guinea pigs tested. The research sample is guinea pig hair that has been shaved on the back and given 6 formulations of hair tonic formulations. All treatment has been approved by the ethics committee

Extraction

Making tea leaf (*Camellia sinensis* L.) and celery leaf (*Apium graveolens*, L.) simplicia powder by choosing tea leaves and celery leaves that are still fresh and green from . Tea and celery leaves then cleaned and washed, drained, dried in the sun for 7 days. Dried tea and celery leaves then powdered and sieved with a 40 mesh sieve. The filtered powder is collected for the extraction process

Tea and celery leaf extract was obtained by maceration methods. Dried simplicia powder was put into a glass jar as much as 900 gr then added 96% ethanol solvent using a ratio of 1:10 (simplicia : ethanol). Macerated for 3x24 hours with every 6 hours stirring. The screening process is at least two screenings over a period of 3 days. The

collected filtrate after 3 days maceration collected and then concentrated using a rotary evaporator at a temperature of 50°C until a concentrated extract is obtained. concentrated extract is ready to be used for the hair tonic formulation process (Hindun et al., 2017).

Formulation of Hair Tonic

Hair tonic formulations were made with 6 formulations, namely negative control, positive control of celery leaf extract, positive control of green tea leaf extract, formulation of 1 green tea leaf 75% and celery leaf extract 25%, formulation of 2 green tea leaves 50% and celery leaf extract 50%, formulations of 3 green tea leaf extracts 25% and celery leaves 75%, hair tonic formulations are presented in table 1. Formulation is based on previous study with addition two formula as positive control (Hindun et al., 2017).

Evaluation of Hair Tonic

Evaluation of hair tonic formulations was carried out by physical evaluation with organoleptic tests by looking at the smell, color and texture of the formulations carried out once every 7 days for 28 days carried out by untrained panelists and pH testing using a pH meter and evaluation of hair growth activity observing the hair growth of guinea pigs. Every 7 days for 28 day and measure it with a caliper (Hindun et al., 2017).

Table 1. Hair Tonic Formulations

Ingredient	Formulation					
	Negative Control	Positive Control green tea leaves	Positive Control celery leaves	F1	F2	F3
Tea Leaf Extracts	-	10	0	2,5	5	7,5
Celery Leaf Extracts	-	0	10	7,5	5	2,5
Ethanol 96%	35	-	-	35	35	35
Propilenglikol	15	-	-	15	15	15
Metil paraben	0,075	-	-	0,075	0,075	0,075
Propil paraben	0,025	-	-	0,025	0,025	0,025
Natrium metabisulfit	0,05	-	-	0,05	0,05	0,05
Mentol	0,2	-	-	0,2	0,2	0,2
Tween 80	1	-	-	1	1	1
Na ₂ EDTA	0,2	-	-	0,2	0,2	0,2
Aquadest	Add100	Add100	Add100	Add100	Add100	Add100

RESULTS AND DISCUSSION

Celery Leaf (Apium graveolens L.) Extract

In the manufacture of extracts, the process of making simplicia is carried out before the extraction is carried out. The amount of celery leaf simplicia produced is 900 grams from 20 kilograms of celery leaves. Celery leaf simplicia was then extracted by maceration method and concentrated with a rotary evaporator. The thick extract of celery obtained has a soft, sticky and thick texture. Celery leaf extract from 900 grams of simplicia resulted in a yield of 34,92 grams (3,88%). Celery (*Apium graveolens L.*) is a plant from the apiaceae family that contain carbohydrates, flavonoids, alkaloids, steroids, limonene, selinene, furocoumarin glycosides, vitamins A and C (Kooti & Daraei, 2017). Phytochemicals screening of

celery leaf extract contain flavonoid, saponin and tannins.

Extraction was carried out using ethanol based on previous research which showed ethanol is a universal solvent that is able to attract all types of active substances, both semi-polar and polar so that active compounds such as flavonoids will be dissolved in ethanol. In addition, the absorption of ethanol is good and the level of toxicity is relatively low to living things (Lase, 2019; Nusmara, 2012).

Green Tea (Camellia sinensis L.) Leaf Extract

Green tea (*Camellia sinensis L.*) is very rich in polyphenolic, flavonols, phenolic, proanthocyanidins (prodelphinidin), xanthic bases (caffeine, theophylline), polysaccharides, essential amino acids, also vitamins (B, C, E), and minerals and trace

elements (calcium, magnesium, manganese, copper, zinc, selenium, potassium (Spadiene et al., 2014) Phytochemicals screening of green tea leaf extract contain flavonoid, saponin and tannins. The resulting green tea leaf extract has a thick and sticky texture. A total of 300 grams of simplicia powder resulted in a green tea leaf extract yield of 34,98 grams (11.66%). The results obtained are much different from the results of Husna's research, (2019) which shows the yield resulting from the extraction process of the green tea leaf maceration method is 19.06%. Thick extracts are formulations that cannot be poured cold. The extract is designed in a viscous form because it lowers the water content of the extract. The high water content in the extract can cause instability of the formulation because it is susceptible to contamination by bacteria (Istiqomah, 2013).

Formulation of Hair Tonic Formulations

Hair tonic formulations for hair growth were made with 6 formulations, a negative control formulation (base), a positive control formulation of 10% green tea leaf ethanol extract, a positive control formulation of 10% celery leaf ethanol extract, a formulation of 1 green tea ethanol extract: ethanol extract 1:3 or green tea leaf ethanol extract 2.5% and celery leaf ethanol extract 7.5%, formulation 2 green tea ethanol extract:ethanol extract 2: or 5% green tea leaf ethanol extract and 5% celery leaf ethanol extract and formulation 3 green tea ethanol extract:extract ethanol 3:1 or ethanol extract of green tea leaves 7.5%

and ethanol extract of celery leaves 2.5%. The percentage of extract used refers to a study by Hindun et al.,(2017) which showed that hair tonic with an extract content of at least 2.5% and a maximum of 10% showed physical stability with storage for 28 days. The content of other ingredients in the formulation such as menthol, ethanol 96%, propylene glycol, sodium metabisulfite, methylparaben, tween 80 and aquadest makes the formulation work optimally because each ingredient has activity as a solvent, preservative, stabilizer, antioxidant, suspending agent and fresh aroma.

Organoleptic Test

Organoleptic tests consist of color, smell and texture. The results of the organoleptic test of hair tonic formulations in 6 formulations are presented in table 2. The organoleptic test was carried out by placing the hair tonic formulation in a container and then observing the color, smell and texture by untrained panelists. The organoleptic test was carried out 5 times with a time span of once a week for 4 weeks, namely on days 0, 7, 14, 21, 28. The results of the organoleptic test showed that the color of the hair tonic formulation, the positive control formulation, was green and the hair tonic formulation was a combination of green tea leaves and celery leaves. brown, based on color for 4 weeks in each formulation, hair tonic formulations showed no color change. Odor organoleptic test showed a characteristic odor and no change in odor during 28 days of storage.

Texture organoleptic test showed that all formulations had a liquid texture and were easy to apply to guinea pig hair. The

appearance of the hair tonic of all formulations can be seen in Figure 1.

Table 2. Organoleptic Test of Hair Tonic Formulations

Organoleptic	Days to	Formulation					
		Negative Control	Positive Control	Positive Control	F1	F2	F3
			green tea leaves	celery leaves			
Color	0	Colorless	Deep Green	Green	Brown	Clear brown	Tea Brown
	7	Colorless	Deep Green	Green	Brown	Clear brown	Tea Brown
	14	Colorless	Deep Green	Green	Brown	Clear brown	Tea Brown
	21	Colorless	Deep Green	Green	Brown	Clear brown	Tea Brown
	28	Colorless	Deep Green	Green	Brown	Coklat Bening	Tea Brown
Smell	0	Aroma therapy	Typical	Typical	Aroma therapy	Aroma therapy	Aroma therapy
	7	Aroma therapy	Typical	Typical	Aroma therapy	Aroma terapi	Aroma terapi
	14	Aroma therapy	Typical	Typical	Aroma therapy	Aroma therapy	Aroma therapy
	21	Aroma therapy	Typical	Typical	Aroma therapy	Aroma therapy	Aroma therapy
	28	Aroma therapy	Typical	Typical	Aroma therapy	Aroma therapy	Aroma therapy
Texture	0	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	7	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	14	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	21	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	28	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid

Organoleptic examination was carried out to observe any physical changes in the formulation, such as color changes, the appearance of odor or not, and texture changes. On texture observation, it is seen that the formulations is homogeneous. Organoleptic observations can also be used to

test the physical stability of the formulation. The formulation can be declared stable if it does not experience a color change in the formulation (Nusmara, 2012). The stability of the formulation is influenced by environmental conditions such as temperature, light and pH. If a formulation is

unstable, both physically and chemically, it can cause a decrease in the pharmacological effect of the active substance in the formulation (Oktami et al., 2021). The stability of the formulation is also influenced by several ingredients such as propylene glycol, glycerin, CMC-Na, antioxidant content. which is able to prevent oxidation such as sodium metabisulfite (Priskila, 2012; Sayuti, 2015).

In this study, hair tonic was declared stable in all formulations, both positive control formulations containing only one ethanol extract of green tea leaves or celery leaves and formulation 1, formulation 2 and formulation 3 which were combination formulations of ethanol extract of green tea leaves and celery leaves. All formulations are stable because the environmental conditions in the storage process are the same, besides that the formulations contain ingredients that

are able to maintain the stability of the formulation such as propylene glycol and sodium metabisulfite.

pH measurement

Measurement of pH using a digital pHmeter. First, the pH meter was calibrated using a neutral pH, then washed with distilled water, then dried using a tissue. Measurement of the pH of the hair tonic formulation is done by dipping the pH meter into the formulation, waiting for a while until the pH meter shows a constant number (Sona, 2018). The results of pH measurements showed that the pH of the formulations in all formulations was in accordance with the pH of the skin, namely 4.5-7.5. pH stability is included in the stable category because during one month of storage, the pH of the formulation does not change much. Details of the pH measurement results for all formulations are presented in table 3.

Table 3. pH Value

Days to	Formulation					
	Negative Control	Positive Control green tea leaves	Positive Control celery leaves	F1	F2	F3
0	5,1	4,3	5,2	5	4,9	4,8
7	5,4	4,3	5,2	5	4,8	4,8
14	5,2	4,3	5,2	5	4,8	4,8
21	5,3	4,3	5,0	4,9	4,8	4,8
28	5,3	4,2	5,0	4,9	4,8	4,8

The pH measurement was carried out to determine the suitability of the pH of the hair tonic formulation with the pH of the skin, which was 4.5-7.5. The pH value of topical formulations should not be too acidic because

acidic pH can cause skin irritation, and also should not be too alkaline, because alkaline pH can cause scaly skin (Mulyanti et al., 2019). The composition of the formulated ingredients, both active substances or additives used affect the pH of the

formulation, changes in the storage pH value indicate a damage to the constituent components in the formulation so that it will affect the resulting effect when applied. Changes in pH can be affected by storage temperature and light from outside. Light is a catalyst for oxidation reactions that can accelerate the oxidation reaction and change the pH value (Dewi et al., 2018).

In this study, pH hair tonic was declared stable in all formulations, starting from the first to the 28th day, this was because the environment in the formulation process was the same temperature and minimal lighting because it was stored in dark bottles and coated with aluminum foil.

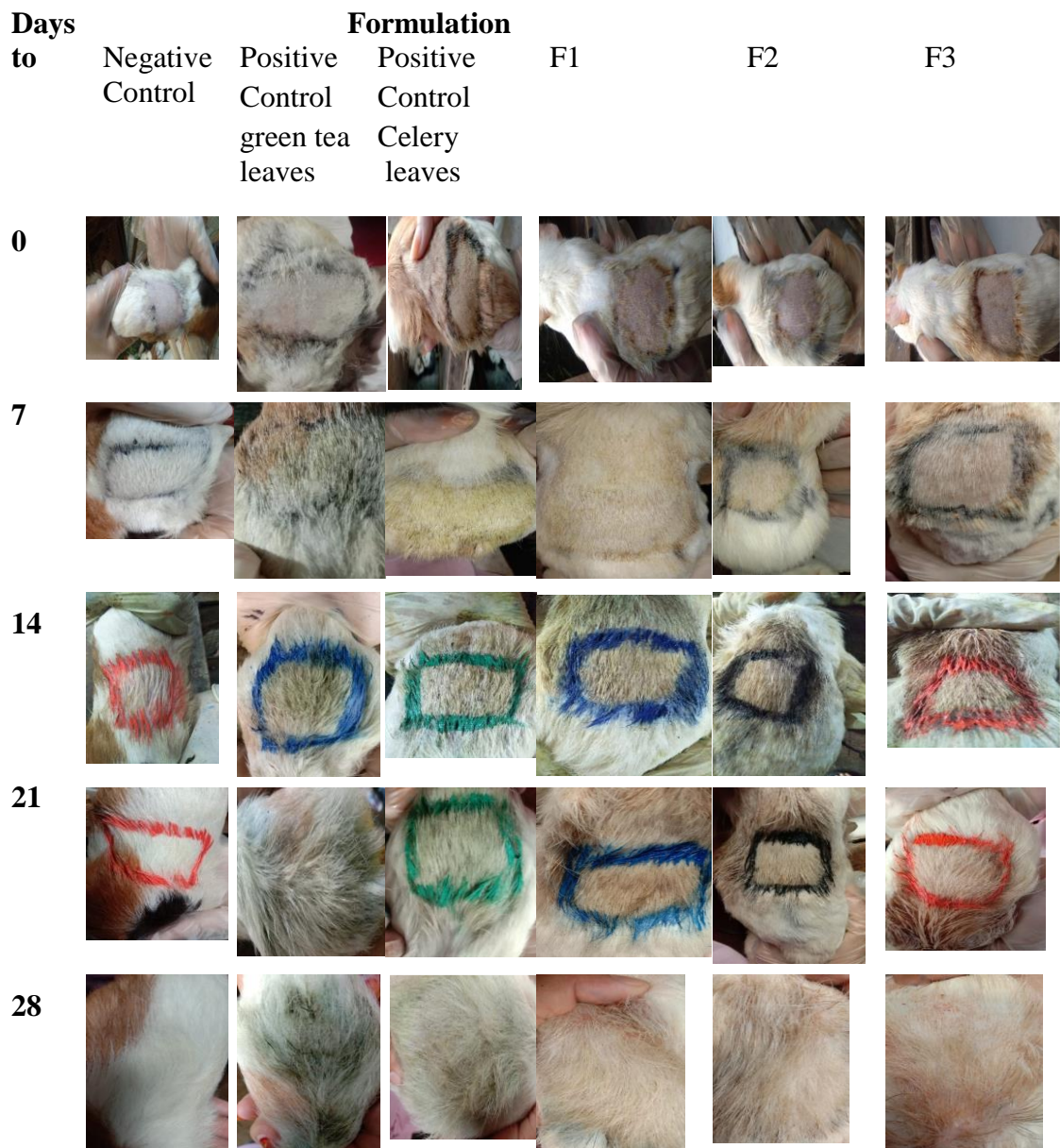


Figure 2. Hair Growth of Guinea Pigs

Guinea Pig Hair Growth Activity Test

Before observing hair growth in guinea pigs, guinea pigs were acclimatized for 1-2 weeks. Acclimatization aims for animals to try to adapt to a new environment. The guinea pigs used were 24 guinea pigs and were divided into 6 groups with 4 guinea pigs in each group. Group 1 received negative control hair tonic treatment, group II received positive control hair tonic treatment with 10% green tea leaf extract, group III received positive control hair tonic treatment with 10% celery leaf extract, group IV received formula 1, group V received formula 2 and Group VI got formula 3. Observation of guinea pig hair growth was done by shaving 3 cm x 3 cm on the back of guinea pigs in all treatment groups, after that the hair tonic formulation was applied to different guinea pig groups for 28 days. Observations were made on the 7th, 14th, 21st and 28th days, every 7 days the guinea pig hair was removed and then measured using a caliper. Administration of the formulation by applying 1 ml every day for 28 days, the first day of application is considered day 0. This study uses guinea pigs because guinea pigs are one of the animals that have good body resistance compared to rabbits, animals that are easy to find, guinea pigs are also animals that easily adapt to new places (Wulandari, 2017).

The results showed that the hair tonic formulation formulated in this study had the ability to grow hair. The growth of the guinea

pig's hair can be seen in Figure 1. From Figure 1 it is known that starting on the 14th day, the guinea pig's hair has started to grow to meet the initial part that was cut. Furthermore, the hair is getting longer and on the 28th day there are no more visible scars on the cut skin at the beginning of the study. This indicates that the hair tonic formulations in all formulations in this study have hair growth activity.

The average hair growth of guinea pigs is presented in table 4. In Table 4, it is known that grow hair faster for guinea pig is found in formulation 1, which is a combination formulation of green tea leaf ethanol extract and celery leaf ethanol extract with a ratio of 1:3, which consists of tea leaf ethanol extract. green 2.5% and ethanol extract of celery leaves 7.5%, with an average hair growth of guinea pigs on day 28 of 18.92 mm. Statistical test of hair tonic formulation in this study used SPSS version 25 application. Normality test was carried out using testshapiro-wilk. The results of the Shapiro-Wilk test showed a sig value > 0.05 which indicated that the hair tonic formulation data was normally distributed. Furthermore, the ANOVA test was carried out to determine whether the hair tonic formulations had the same or different averages, the results of the ANOVA analysis showed that there were significant similarities to the average hair growth in each treatment. green tea leaf and celery leaf ethanol extract on hair growth activity test in guinea pigs. In the paired

sample T-Test test if the value of Sig. (2-tailed) < 0.05 which means that the combination of celery leaf ethanol extract and green tea leaf ethanol extract has hair growth activity in guinea pigs.

The combination of green tea leaf ethanol extract and celery leaf ethanol extract has hair growth activity. When compared with the positive control, formulas 2 and 3 had a smaller effect on hair growth, while formulation 1 had the same effect and or better than the positive extract. These results are the same as those of Hindun et al. (2017) which showed a formula with 2.5% green tea leaf extract and 7.5% celery leaf extract had better activity than positive control. Green tea leaves contain flavonoids that can increase the rate of hair growth. Hair tonic formulations containing water fraction containing flavonoids from ethanol extract of green tea leaves have hair growth activity and do not cause irritation by irritation test on skin (Noviani et al., 2019). Celery leaves are known to contain apiin which is a flavonoid glycoside that undergoes hydrolysis to become the aglycone apigenin. Apiin has vasodilator activity, one of which can stimulate hair growth. The results of qualitative examination, ethanol extract of celery leaves contain flavonoids. In addition to apiin, the amino acid content in celery leaves also helps in the formation of protein which is the main substance for building hair (Jubaidah et al., 2018). Seeing the hair growth ability of green tea leaf ethanol

extract and celery leaf ethanol extract, a hair tonic formulation was developed with a combination of green tea leaf ethanol extract and celery leaf ethanol extract. The combination is done in the hope of increasing the ability of hair growth in hair tonic formulations. In this study, the formula with a higher percentage of green tea leaf ethanol extract had a greater hair growth ability than the formula with the same or less percentage of green tea leaf extract. And when compared with the positive control, green tea leaf extract also showed better results. This happens because formula 1 contains the most effective active substances for growing hair, but it still needs to be tested qualitatively and quantitatively for its active substance content.

CONCLUSION

Green tea leaves (*Camellia sinensis* L) extract, celery leaves (*Apium graveolens* L), and the combination of green tea leaves (*Camellia sinensis* L) extract and celery leaves (*Apium graveolens* L) extract can be formulated and used as hair growth in guinea pigs. The best grow hair fast formulation is combination of green tea leaves extract : celery leaves extract is 2,5%:7,5%.

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