

## Hair Growth Activity of Hair Tonic Preparations Containing Cinnamon Essential Oil (*Cinnamomum burmanii*) and Virgin Coconut Oil

### (Aktivitas Penumbuh Rambut Sediaan Hair Tonik Minyak Esensial Kayu Manis (*Cinnamomum burmanii*) and Minyak Kelapa)

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**Abstract:** Cinnamaldehyde in cinnamon essential oil, lauric acid, myristic acid, and oleic acid in virgin coconut oil (VCO) were used in hair tonic preparations as hair growth agents. This study aimed to evaluate particle size and obtain optimum formulation of the hair tonic preparation and to observe growth activity with various levels of cinnamon essential oil and VCO. This research an experimental with rabbit used 4 test were divided into 6 treatment groups: 1) positive control (Regrou®); 2) normal control (without treatment); 3) negative control (hair tonic basis); 4) F1 (1% cinnamon essential oil: 2.5% VCO); 5), F2 (3% cinnamon essential oil : 2.5% VCO); and 6), F3 (5% cinnamon essential oil: 2.5% VCO). The particle size analysis selected in formula 2 that cycle stable during storage, and fall into the microemulsion size range of 20-200 nm and there was no difference cycle because the significance was >0.05. The hair growth activity was determined using Average Growth Daily Gain (AGD) formula and then was analyzed statistically using One Way Anova. The results showed that had hair growth activity that was not significantly different. However, when compared with the positive control, formula 3 has hair growth activity above the positive control.

**Keywords:** AGD, cinnamaldehyde, essential oil, hair growth.

**Abstrak:** Sinamaldehyd pada minyak atsiri kayu manis dan kandungan asam laurat, asam miristat, asam oleat pada VCO (*virgin coconut oil*) dapat digunakan sebagai penumbuh rambut. Penelitian ini bertujuan untuk melihat ukuran partikel, aktivitas pertumbuhan rambut dari sediaan hair tonik dengan variasi kadar minyak atsiri kayu manis dan VCO, serta mendapatkan konsentrasi yang optimal. Penelitian ini bersifat eksperimental dengan enam kelompok perlakuan diantaranya kontrol positif (Regrou®); kontrol normal (tanpa perlakuan); kontrol negatif (basis hair tonic); F1 (1% minyak atsiri kayu manis: 2,5% VCO); F2 (3% minyak atsiri kayu manis: 2,5% VCO); dan F3 (5% minyak atsiri kayu manis: 2,5% VCO). Analisis ukuran partikel dipilih pada formula 2 dimana menunjukkan siklus stabil selama penyimpanan, dan masuk dalam rentang ukuran mikroemulsi 20-200 nm yang menunjukkan tidak ada perbedaan signifikan (>0.05) pada siklus. Data aktivitas pertumbuhan rambut dihitung menggunakan rumus AGD (*Average Growth Daily Gain*) kemudian dianalisis secara statistik menggunakan ANOVA satu arah. Hasil penelitian menunjukkan bahwa hair tonik kombinasi minyak atsiri kayu manis dengan VCO pada F1, F2, dan F3 tidak memiliki perbedaan yang signifikan terhadap aktivitas pertumbuhan rambut pada kelinci. Secara AGD kemampuan pada F2 dan F3 sebagai penumbuh rambut menunjukkan nilai di atas kontrol positif.

**Kata kunci:** AGD, minyak esensial, penumbuh rambut, sinamaldehyd.

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## INTRODUCTION

THE MOST common hair problem is hair loss, which can lead to baldness. A treatment for repairing the hair loss is by using hair tonic<sup>(1)</sup>. Natural ingredients are currently more preferred because they are safe and relatively have lower side effects compared to the synthetic ones<sup>(2)</sup>. Cinnamon essential oil and virgin coconut oil (VCO) are the herbal ingredients that can be potentially used for hair care. Cinnamon essential oil contains an antioxidant compound, namely cinnamaldehyde<sup>(3)</sup>. Antioxidants have potential activity in reducing hair loss<sup>(4)</sup>. Previous research has reported that cinnamon essential oil has been proven to grow hair at a concentration of 100%<sup>(5)</sup>. Utilisation of other products includes virgin coconut oil (VCO). Virgin coconut oil contains saturated fatty acids such as lauric acid, myristic acid, and oleic acid which has also effective to decreases hair loss. In previous research, the use of VCO at a concentration of 100% was demonstrated to accelerate the hair growth of male rabbits<sup>(6)</sup>.

Therefore, incorporation of cinnamon essential oil and VCO in hair tonic preparations is expected to have synergistic activity as a hair growth. The hair tonic preparations is intended for easy use, does not leave deposits and is not sticky when applied to the scalp<sup>(7,8)</sup>. The formula of a hair tonic requirements i.e. organoleptic, homogeneity, pH and viscosity<sup>(9)</sup>. Hair tonic preparation can be made by microemulsions. Microemulsions are nanometer-sized droplet dispersions of homogenous liquid solutions<sup>(10)</sup>. The microemulsion system consists of water, oil and amphiphile, which is isotropic and thermodynamically stable and has droplet sizes ranging from 20-200 nm<sup>(11)</sup>. Microemulsions are able to improve permeation of lipophilic and hydrophilic compounds that are suitable for application of hair growth formula<sup>(12)</sup>.

Based on the description, this study aimed to obtain optimum formulation hair tonic preparation incorporated with combination of cinnamon (*Cinnamomum burmanii*) essential oil and VCO using a microemulsion system. The activity of the microemulsion preparations were observed by testing them to rabbits.

## MATERIALS AND METHODS

**MATERIALS.** The materials used in hair tonic preparation were cinnamon essential oil produced by Nusaroma (*Cinnamomum burmanii*), virgin coconut oil (Coco Indo Jaya, Indonesia), alcohol 96% (PT. Kreasi Dua Saudara, Indonesia), methyl paraben, menthol (PT. Brataco, Indonesia), dexpanthenol (Herbal Gayatri, Indonesia), polyoxyethylene 40 hydrogenated castor oil (Zona Kimia, Indonesia),

propylene glycol (Zona Kimia, Indonesia), tween 80 (Raja Kimia, Indonesia), aquadest, buffer pH 4 and 7. For sample testing, the preparations were subjected to rabbits (Wistar).

**Equipments.** The tools used included glass tools (Pyrex, USA), mixer (Maspion, Indonesia), electric stove (Maspion, Indonesia), analytical balance (Ohaus, USA), viscometer Ostwald (Pyrex, USA), pH meter (Handylab pH11/SET SI Analytics, Indonesia), pycnometer (Herka, USA), and particle size analyzer (HORIBA SZ-100 for Windows Z type, France).

**METHODS. Preparation of Hair Tonic.** Three different formulations (1%, 3%, and 5%) of hair tonic preparations containing combination of cinnamon essential oil and VCO were distinguished based on various concentrations of the cinnamon essential oil according to previous research<sup>(13)</sup>. Tween 80 and polyoxyethylene 40 hydrogenated castor oil were dissolved in hot distilled water, while propylene glycol was dissolved in distilled water. They were mixed homogeneously using a magnetic stirrer. Methyl paraben, menthol, and d-phenol were dissolved in alcohol 96% using stirrer. The oil phase consists of cinnamon essential oil and VCO was also mixed and put in a mixer and made up to the appropriate volume.

**Physical Characterization.** Organoleptic test were examined by colour observation of the hair tonic preparations was carried out on a white background or base in the panellist room, equipped with bright light. Observation on aroma was performed by inhaling the aroma of the formula, and the texture was determined by pouring the samples on a slideglass and observed.

Homogeneity test was carried out by observing the presence of coarse grains found in the preparation mounted on a slideglass. The hair tonic was gently dripped over the clean slideglass, to observe for the presence of grains. Hair tonic without granules were determined as homogeneous<sup>(7)</sup>.

Determination of the pH of the hair tonic preparation was measured using a digital pH metre (Handylab pH11/SET SI Analytics). The electrodes were dipped in a glass beaker containing hair tonic and the pH values were recorded<sup>(14)</sup>.

The density testing was carried out using a pycnometer (Herka) at a room temperature of 25°C. The initial empty pycnometer was weighed (W0), was then filled with aquadest and weighed (W1). Furthermore, the specific gravity test was carried out for each hair tonic sample. The pycnometer was firstly cleaned and the sample was inserted gently to prevent air bubbles and again the pycnometer was then weighed (W2). The density of each sample was measured using the below equation (1):

$$\text{Density} = \frac{W2 - W0}{W2 - W1}$$

Determination of the hair tonic viscosity preparations using an Ostwald viscometer (Pyrex). A 10 ml of hair tonic preparation was put in a large tube (A), the hair tonic was then sucked using a filler pump until it exceeded the limit of line 1 (b). The hair tonic flow was observed at the boundary line 1 (b) and line boundary 2 (a) while measuring the flow- rate of hair tonic using a stopwatch<sup>(16)</sup>.

**Particle Size Analysis.** The particle size analysis (PSA) (HORIBA SZ-100 for Windows Z type) was preheated for  $\pm 20$  minutes before running the samples. The first procedure, a standard solution of 20 dilutions was shaken using a vortex mixer for  $\pm 1$  minute, then put in a clean cuvette until it was filled with 2/3 cuvette. The cuvette is inserted into the device and closed with a sensor, then the temperature is conditioned at 25°C by pressing the "Temp Panel" menu. The standard starts to be measured by pressing the "Auto1" menu, then the tool will automatically measure the size of the particle. The next procedure uses an automatic method with a sharp distribution form. The second procedure is carried out in the same way as the first procedure, but the distribution graph settings are replaced with sharp shapes<sup>(17)</sup>.

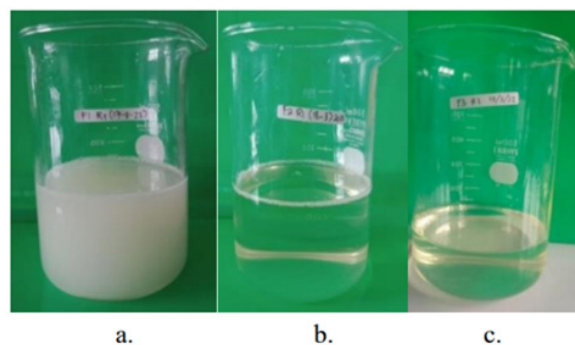
**Data Analysis.** The collected data of organoleptic and homogeneity test, pH, density, and viscosity were analysed using simple linear regression with a significant value at  $P < 0,05$ . Data of the particle size were statistically analysed using a Wilcoxon Test, while data of the hair length were determined using and average growth daily gain (AGD) (equation 2) calculation followed by One Way ANOVA analysis. Data processing was performed using SPSS software.

$$\text{AGD formula} = \frac{\text{Average Hair Length day 18} - \text{Average Hair Length Day 3}}{(18-3)} \quad (2)$$

## RESULTS AND DISCUSSION

**Physical Characterization.** The organoleptic of hair tonic combination of cinnamon essential oil (*Cinnamomum burmanii*) with VCO (virgin coconut oil) has a slightly thick liquid dosage form (Figure 1), with different colours and aromas produced between formulas, the higher the concentration of cinnamon essential oil, the clearer and more fragrant the aroma will be strong in the resulting formula 3 preparation. The increase in the stronger aroma occurs due to the presence of the compound cinnamaldehyde which is the largest compound in cinnamon essential oil<sup>(18)</sup>.

The homogeneity test on the three hair tonic formulas showed that there were no coarse grains in the object glass that was dripped with hair tonic.



**Figure 1. Organoleptic formula 1 (a), formula 2 (b), and formula 3 (c).**

This shows that the three hair tonic formulas have a homogeneous composition. Homogeneous hair tonic preparations are caused by the long stirring during the mixing process, the stirring time can increase the contact time between the ingredients so that the preparation becomes homogeneous<sup>(19)</sup>.

pH test is carried out to determine the acidity level of the hair tonic, it will cause irritation when applied to the scalp. Acidic pH value of hair tonic will cause irritation and if it is too alkaline it can cause coconut skin to become scaly<sup>(20)</sup>. The pH value of the three hair tonic formulations showed in the range of 3.0-7.0, it can be concluded that the hair tonic has suitable pH for the scalp and hair tonic pH requirements. Cinnamaldehyde in cinnamon essential oils has an acidic pH of 4.7<sup>(18, 21)</sup>. It is concluded that the higher concentration of cinnamon essential oil resulted in a decrease in the pH value sequentially from F1, F2, and F3 due to the increasing amount of cinnamon essential oil in the preparation (Table 1).

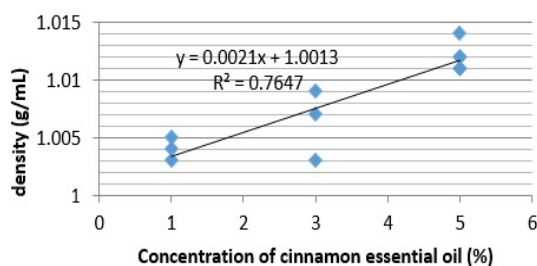
Cinnamon essential oil has several ingredients, such as 3%-8% cinnamic acid and 68.3%-82% cinnamaldehyde. Cinnamaldehyde in cinnamon essential oil has an acidic pH of 4.7<sup>(18, 21)</sup>. It can be explained that the higher the concentration of cinnamon essential oil used, the lower the pH value.

Density is the ratio between mass and density of water at a certain temperature. It can describe the ease of preparation of hair tonic to be poured. The type of liquid dosage form is good if it has a value close to the density of water<sup>(22)</sup>. The results of the measurement of density on the three hair tonic formulations showed an increase in specific gravity along with an increase in the concentration of cinnamon essential oil (Figure 2). A hair tonic is said to have a good density if it has a specific gravity value close to water. Water has a density of 0.99713 at a temperature of 25°C<sup>(23)</sup>. The density of all formulas showed values that were close to water which indicated that the hair tonic preparation was easy to pour. Based on SNI 06-3734-2006, cinnamon essential oil has a density at 20°C of 1.008-1.030. The conclusion, increasing the concentration

**Table 1. pH test of hair tonic.**

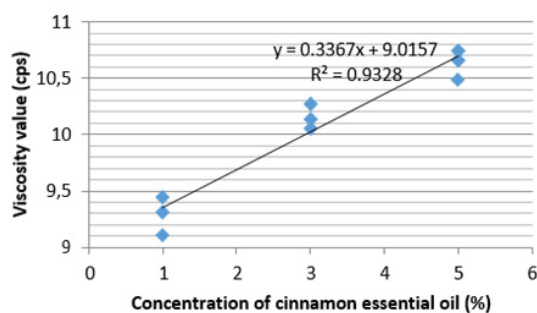
Concentration	pH range			Average $\pm$ SD
	Replication 1	Replication 2	Replication 3	
1 %	6.02	6.09	6.06	6.05 $\pm$ 0.035
3 %	5.95	5.95	5.90	5.93 $\pm$ 0.028
5 %	5.60	5.75	5.75	5.70 $\pm$ 0.086

of cinnamon essential oil will increase the value of specific gravity of hair tonic preparations. Therefore, it can be concluded that cinnamon essential oil can affect the density of hair tonic preparations (Figure 2).



**Figure 2. Curve of specific gravity value of hair tonic preparation.**

Viscosity test was conducted to determine the resistance of the hair tonic combination when flowing. The higher value of the solution viscosity, resulting thicker solution. Thick hair tonic will cause crust on the scalp that can cause dandruff. A good hair tonic has a low viscosity, that it produces a liquid preparation<sup>(16)</sup>. The hair tonic viscosity test was carried out using an oviscometer because the liquid hair tonic dosage form was slightly thick. Viscosity values in the three hair tonic formulations showed an increase in viscosity along with increasing variations in the concentration of cinnamon essential oil (5% concentration). Based on the research of<sup>(24)</sup> the viscosity of the hair tonic of eucalyptus essential oil increased along with the increase in the concentration eucalyptus in the concentration of eucalyptus essential oil. So that the research is in accordance with the reference, namely the higher the concentration of cinnamon essential oil, the higher the viscosity value sequentially from F1, F2, and F3 (Figure 3).



**Figure 3. Viscosity value curve for hair tonic preparation.**

**Particle Size Analysis Test.** Based on the physical stability test of the essential oil combination hair tonic preparations of cinnamon with VCO it can be concluded that formula 1 (1%) has a cloudy white colour, smell of cinnamon, homogeneous, and physically unstable because the pH and viscosity showed a significant difference in results during storage. Formula 2 (3%) has a clear colour, a characteristic odor of wood sweet, homogeneous, and stable during storage, while Formula 3 (5%) has a clear colour, a distinctive cinnamon odour, homogenous and physically unstable because pH show the result that there is a difference physically meaning during storage. Hair tonic in Formula 2 (F2) with a concentration of 3% cinnamon essential oil was chosen to be tested for particle size because it has good physical stability for 6 cycles. These results indicate that hair tonic formula 2 with a concentration of 3% cinnamon essential oil falls into the microemulsion size range of 20-200 nm. The particle size results were analysed using the wilcoxon test and showed that there was no difference between cycle 0 and cycle 6 because the significance was 0.05.

The polydispersity index (PI) value is used to describe the variation in the sample. If the value of the polydispersity index is small, that is  $<0.1$ , it indicates that the sample is monodispersion. The polydispersity index value in F2 cycle 1 is replication 1 0.335 and replication 2 0.448. While the value of Polydispersity index F2 in cycle 6, namely replication 1 0.453 and replication 2 0.465. The results of the polydispersity index value in F2 cycles 0 and 6 showed that the level of uniformity of globulesize in the microemulsion was still low because  $<0.1$ . The results of the Polydispersity index were analysed using the wilcoxon test statistic and showed that there was no difference between cycles 0 and 6 because the significance was 0.05.

**Hair Growth Activity Test.** Ethical clearance with no 7/I/2022/Bioethics commission has been issued by the Medical/Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung University. The animals were acclimatized for 1 week. The hair on the back of each rabbits was shaved, then divided into 6 areas, each  $3 \times 3$  cm<sup>2</sup>, and with distance between areas of approximately 1 cm.

The average hair length diagram can be seen in Figure 4. Based on the data, it can be seen that the normal control had the smallest average hair length of 1.11 mm on the 3<sup>rd</sup> day and 5.13 mm on the 18<sup>th</sup> day. The third treatment group, formula 3 (5% cinnamon



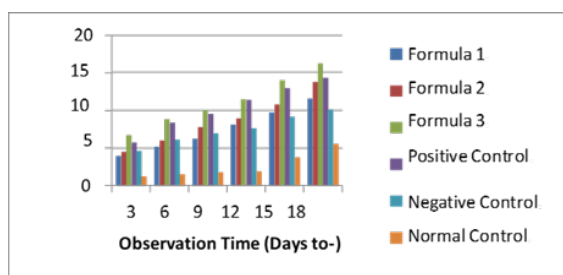


Figure 4. Rabbit hair length chart.

essential oil: 2.5% VCO) had the highest mean hair length, which was 5.93 mm on the 3<sup>rd</sup> day and 14.54 mm on the 18<sup>th</sup> day. The results of the average hair length have not been able to explain the actual situation regarding how big the effect of giving a hair tonic combination of cinnamon essential oil with VCO on rabbit hair growth per day. Therefore, the calculation is carried out using the average growth daily gain (AGD) formula. The existence of these calculations can provide an explanation that is closer to the actual situation regarding the effect of giving a hair tonic combination of cinnamon essential oil with VCO on rabbit hair growth per day.

The diagram of the average hair length per day based on the AGD calculation can be seen in Figure 5. The results of the hair tonic activity test for the combination of cinnamon essential oil with VCO based on the AGD calculation show that hair tonic formula 3 (5% cinnamon essential oil: 2.5% VCO) has the largest mean AGD value, which is 0.58 mm. Based on these results, it can be said that the hair tonic combination of 5% cinnamon essential oil with 2.5% VCO has a hair growth speed of 0.58 mm per day. Where as shown in table I formula 3 has the highest concentration of essential oils compared to other formulas. Meanwhile, the average AGD hair tonic formula 2 (3% cinnamon essential oil: 2.5% VCO) and formula 1 (1% cinnamon essential oil: 2.5% VCO) respectively were below formula 3 (5% VCO), cinnamon essential oil: 2.5% VCO) ie 0.51 mm and 0.48 mm.

Positive control is needed in this study to determine whether the hair tonic preparation of a combination of cinnamon essential oil with VCO has the same hair growth activity as the positive control. The positive control used was Regrou® hair tonic containing 2% minoxidil because it has been used as a hair growth medicine for more than 20 years<sup>(25)</sup>. The average positive control AGD value was 0.52 mm, so it can be said that positive control could accelerate rabbit hair growth by 0.52 mm per day. Formula 3 has an average AGD value that is greater than the positive control, while formula 1 and formula 2 have an average AGD value that is smaller than the positive control. The negative control was used to determine whether the hair tonic preparation base also had hair

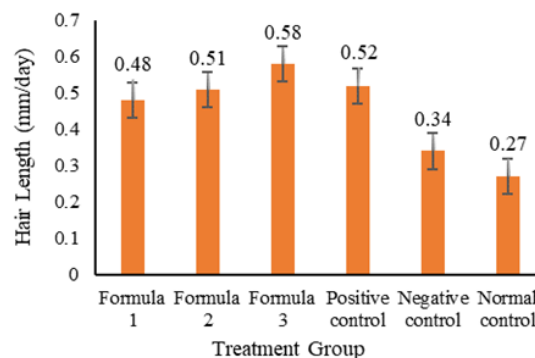


Figure 5. Long mean chart hair per day based on AGD calculation.

growth activity. The negative control had an average AGD value of 0.34 mm, so it could be interpreted that the negative control could accelerate the rabbit's hair growth by 0.34 mm per day. Formula 1, formula 2, and formula 3 have an average AGD value greater than the negative control.

Normal control (without treatment) was used to ensure that the results of hair growth activity in the treatment group were the result of the effect of the treatment. Normal control has the smallest mean AGD value of 0.27 mm, so it can be interpreted that normal control has a rabbit hair growth rate of 0.27 mm per day. The small AGD value obtained by the normal control group was due to the absence of an active compound that can accelerate hair growth, so that hair grows scientifically. Formula 1, formula 2, and formula 3 have an average AGD value greater than the normal control.

Hair length measurement data based on AGD calculations was then analysed using statistics. This is to determine the differences in hair growth activity in each treatment group. Based on statistical analysis using One Way Anova, a significance value of 0.271 ( $> 0.05$ ) was obtained. Therefore, it can be interpreted that there is no significant difference between the treatment group that was given a hair tonic preparation of a combination of cinnamon essential oil and VCO and the treatment group that was given a positive control. Based on this analysis, it can be said that the preparation of hair tonic formula 1 (1% cinnamon essential oil: 2.5% VCO); formula 2 (3% cinnamon essential oil: 2.5% VCO); and formula 3 (5% cinnamon essential oil: 2.5% VCO) had hair growth activity that was not significantly different. However, when compared with the positive control, formula 2 and formula 3 has hair growth activity above the positive control.

The comparison of the concentration of cinnamon essential oil with the VCO used was determined based on the trial and error method because there has been no previous research using cinnamon essential oil in hair

growth preparations. The research results of (5) stated that giving cinnamon essential oil a concentration of 100% could accelerate the hair growth of rats. Meanwhile, the results of Blegur and Indrawati's research (2015) state that VCO at a concentration of 100% can accelerate hair growth more optimally compared to emulsion preparations containing VCO at concentrations of 50% and 75%. Due to the high concentration of cinnamon essential oil and VCO which can accelerate hair growth, the researchers suspect that the ratio of the concentration of cinnamon essential oil to the VCO used needs to be increased.

### CONCLUSION

Cinnamon essential oil can affect the physical characteristics of hair tonic preparations. The particle size results were analysed using the wilcoxon test and showed that there was no difference cycle because the significance was 0.05. Hair growth activity test for hair tonic preparations of a combination of cinnamon essential oil with VCO, it was concluded that preparation of hair tonic formula 1 (1% cinnamon essential oil: 2.5% VCO); formula 2 (3% cinnamon essential oil: 2.5% VCO); and formula 3 (5% cinnamon essential oil: 2.5% VCO) had hair growth activity that was not significantly different. However, when compared with the positive control, F2 and F3 has hair growth activity above the positive control.

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