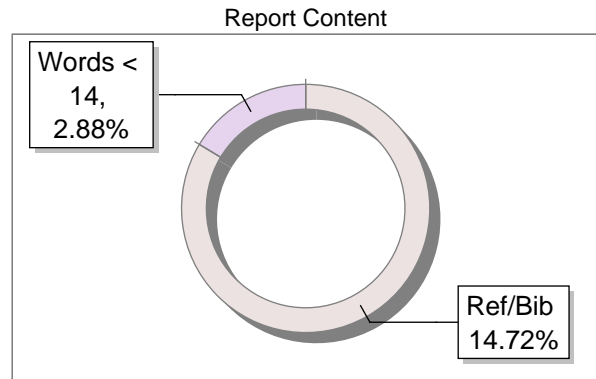
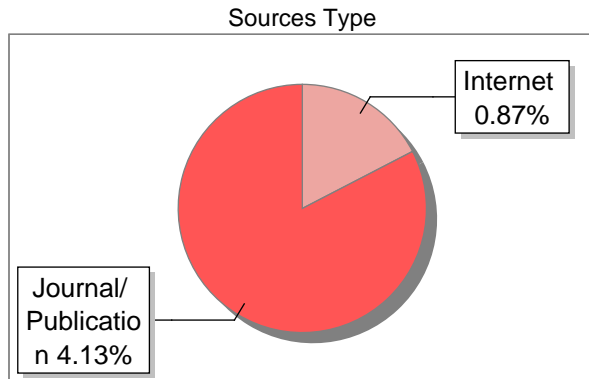
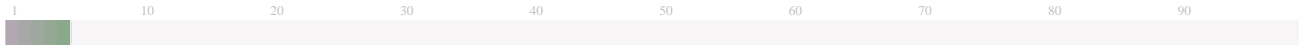


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## Nanoemulsion Formulation Combination of Virgin Coconut Oil (VCO) and Candlenut Oil (*Alleurites mollucanus*) for Hair Growth in Male White Rats (*Rattus novergicus*)

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### ABSTRACT

Having healthy and thick hair is the dream of many people, but some people have problems related to their hair. Hair problems that often occur are hair loss, thin hair or baldness, so to overcome these problems hair growth products have been made. Virgin Coconut Oil VCO and candlenut oil can be used as natural ingredients that can grow hair. The aim of this research was to determine the best nanoemulsion formulation for a combination of candlenut oil and VCO; and to determine the difference between applying nanoemulsion, conventional candlenut oil, conventional VCO and the oil emulsion on hair growth in male rats. This nanoemulsion formulation uses a combination of candlenut oil and VCO in a ratio of 1:1, 1:3 and 3:1. The nanoemulsion obtained was subjected to organoleptic, pH, viscosity, flow type and % transmittance. The best formulation was then tested for droplet size and <sup>16</sup>a potential as well as hair growth activity tests using male rats. It was found that the best nanoemulsion formula was a nanoemulsion with a combination of 1:3 with a yellow color, a distinctive odor and clear with a droplet size of  $14.42 \pm 0.03$  nm, a zeta value of  $-27.8 \pm 1.02$  mV, the length of the hair produced in the test hair growth activity was  $17.14 \pm 0.42$  mm and hair weight was  $699.37 \pm 7.91$  mg.

## INTRODUCTION

<sup>16</sup>Hair is a part of the human body that plays an important role in human life because it is the crown of pride for women and men. Having healthy and thick hair is the dream of many people. However, some people have problems related to their hair, such as hair loss, thin hair and even baldness.

Currently, many products have been formulated to overcome these hair problems, with the main ingredient used being the synthetic ingredient minoxidil. However, the use of minoxidil has been reported to have undesirable side effects such as scalp irritation, hypotension, dizziness and heart palpitations

(Nestor et al., 2021). So there is a need for other alternatives to overcome this problem. The use of natural ingredients can be used as an alternative treatment in this case.

<sup>2</sup>For generations, Indonesian people have used Virgin Coconut Oil (VCO) and candlenut oil which are believed to accelerate hair growth and strengthen the roots and provide a shiny effect when used. Research has been carried out regarding hair growth activity tests using VCO, one of which used a VCO emulsion on rabbits and it was found that 100% VCO emulsion had maximum results in accelerating hair growth in male rabbits (Blegur & Indrawati 2015),

VCO has high antioxidant activity as stated by Ghani et al., (2018) that the IC50 value of VCO is 7.49 - 104.52 mg/mL. Clinical trials related to antioxidant activity for hair have been conducted and it was found that the use of functional antioxidants and barrier-enhancers can further improve the condition of the scalp and allow the reduction of hair loss (Davis et al., 2021).

The main components of VCO, namely around 92%, are saturated fatty acids including lauric acid (48.74%), myristic acid (16.31%), caprylic acid (10.91%), capric acid (8.10%) and caproic acid (1.25%) (Wijayanti et al., 2017) which is included in the medium chain fatty acid group, fatty acid therapy is associated with reducing baldness (Hamel et al., 2017). The antioxidant properties of oleic acid contained in coconut oil can also slow down hair fall and accelerate hair growth (Kartika Sari & Wibowo, 2016).

Apart from VCO, the type of oil that Indonesian people also believe can grow hair for generations has been candlenut oil. The components contained in candlenut oil was found to be 91.77% consisting of oleic acid, linoleic acid and linolenic acid (Yuliani et al., 2018). Linoleic acid has potential as an alternative treatment for hair loss. Therapy using linoleic acid can activate Wnt/ $\beta$ -catenin signaling and induce the growth of human follicles dermal papilla cells (HFDPC) by increasing the expression of cell cycle proteins such as cyclin D1 and cyclin-dependent kinase 2. Linoleic acid significantly inhibits the expression of Dickkopf-related protein (DKK -1), which is the signaling of primary alopecia by dihydrotestosterone (Ryu et al., 2021).

Linoleic acid, oleic acid and linolenic acid are omega 3, 6 and 9 groups that when combined with VCO which has high antioxidant activity will form a good formula for hair growth. This is based on research conducted by Le Floc'h et al., (2015) who studied the provision of omega 3 and 6 supplements as well as antioxidants for 6 months efficiently can reduce hair fall by increasing hair density and reducing the percentage of telogen and minimization proportion in anagen hair.

One of the methods that can be used in formulating active oil-based preparations is nanoemulsions. Nanoemulsions are suitable for the efficient delivery of active substances

through the skin. The large surface area of the emulsion system allows for rapid penetration of active substances. They are non-toxic and do not cause irritation so they can be easily applied to skin and mucous membranes (Chime et al., 2014).

Previous research only aims to determine the activity of each oil for hair growth so the objective of this research is to know the use of combination of the oil by using nanoemulsion method to increase the absorption of the oil. And also to determine the ratio of hair growth activity and hair weight of each oil to nanoemulsion.

## RESEARCH METHODOLOGY

### Equipments And Materials

The materials used in this research were candlenut oil, virgin coconut oil, Tween 80 (Sigma-aldrich), PEG 400 (Alpha Chem Products), Aquadestilata, NaOH (Emsure), methanol (emsure), n-hexane (emsure), NaCl (Emsure), BF3 (Sigma-Aldrich). The equipments used in this research were a stir bar, measuring cup (Pyrex®), beaker (Pyrex®), Gas Chromatography-Mass Spectrometry (GC-MS) Shimadzu, volume pipettes, pH meter, drop pipettes, UV-Vis Spectrophotometer, Particle Size Analyzer, magnetic stirrer.

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Compound Content

Candlenut oil and VCO were not volatile compounds, so before reading the compounds using GC-MS, derivatization is carried out first. First, a NaOH solution is made by mixing 800 mg solid NaOH in 100 mL methanol. Then 200  $\mu$ L of sample was added to 1 mL of n-hexane and 200  $\mu$ L of the NaOH solution in methanol that had been made previously and then heated for 10 minutes while shaking. The mixture was then added with 1.5 mL of BF3 solution and heated for 10 minutes. The mixture was cooled and 1.5 mL of saturated NaCl was added to precipitate the fatty acids and then vortexed for 10 minutes. The supernatant formed was taken and read using GC-MS (Sudjadi & Rohman, 2016) to conduct qualitative test to determine its contents.

### Formulation of Nanoemulsion

This research was carried out experimentally by formulating a nanoemulsion using Tween 80

and PEG 400 base with a ratio of 1:2 and also a combination of VCO: Candlenut oil (1:1, 1:3, 3:1) (Table 1)

**Table 1. Formulation Of Nanoemulsion**

Ingredients	Formulation			
	F1	F2	F3	F4
Candlenut Oil	2.5	3.75	1.25	-
VCO	2.5	1.25	3.75	-
Tween 80	36	36	36	36
PEG 400	18	18	18	18
Distilled water	Add 100	Add 100	Add 100	Add 100

Nanoemulsion was made by mixing the oil phase, namely tween 80 and a combination of active ingredients (VCO and candlenut oil) using a magnetic stirrer for 10 minutes at a speed of 600 rpm, then PEG 400 was added and homogenized again using a magnetic stirrer for 10 minutes at a speed of 600 rpm. Distilled water was added little by little until the volume of nanoemulsion reaches 100 ml while stirring for 10 minutes. The nanoemulsion was then prepared using formulas 2, 3 and 4 by repeating the nanoemulsion formulation process with formulas 2, 3 and 4.

## Evaluation of Nanoemulsion

### Organoleptic test

Organoleptic testing is carried out visually by observing the shape, odor and color of the preparation.

### pH Test

pH testing is carried out using a digital pH meter test equipment that has been calibrated first and the reading taken 3 times.

### Transmittance

Percentage of transmittance was carried out using a UV-Vis spectrophotometer by dissolving 100 µL of nanoemulsion into 5 mL of distilled water which was homogenized with the help of a vortex mixer for 1 minute and the absorbance was measured at a wavelength of 650 nm with a distilled water blank.

An absorbance value close to 100% indicates that the size of the dispersion droplets produced by the nanoemulsion has reached the nanometer size, which is visually visible from the

transparency of the system formed (Senapati et al., 2016).

### Viscosity and flow type tests

To test the viscosity and flow type of nanoemulsion preparations, a Rheosys Merlin VR viscometer with a 25 mm Concentric Cylinders spindle was used at a speed of 10-100 rpm.

### Droplet size test and potential zeta test

Testing droplet size and zeta potential using the Particle Size Analyzer (PSA) at the Pharmaceutical Sterile Preparations Laboratory, Gadjah Mada University (UGM).

### Hair growth activity test in rats

Ethical approval for animal experiments (rats) in this study was obtained from the Ethics Committee of Ahmad Dahlan University with number 012311284. The experimental animals used were 30 male white rats weighing 200 grams. Before use, the experimental animals were acclimated for one week. Thirtyrats were divided into 6 groups so that each group had 5 rats. After that, each rat had its hair shaved off using a hair clipper. Each group was given a different formula: group 1 was given emulsion (consist the same active substance as nanoemulsion), group 2 was given conventional candlenut oil, group 3 was given conventional VCO, group 4 was given F4, group 5 was the positive control group and group 6 was given nanoemulsion.

Nanoemulsion was administered by applying 0.5 mL to the surface of the rat's skin and spreading it evenly while giving a short massage. The hair length of each rat in every experimental group was measured weekly. Treatment of experimental animals was carried out once a day and lasted for 28 days. Determination of hair growth in rats was carried out by measuring the growing hair using a caliper and observing its growth on days 0, 7, 14, 21 and 28. Hair weight or thickness was measured on the 28th day by shaving all the hair in each area tested and then weighing it using a digital scale (Shoviantari et al., 2019). Statistical tests were carried out using SPSS version 29.0.2.0.

## RESULT AND DISCUSSION

## Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Compound Content

The compound content between candlenut oil and VCO is different even though there are compounds that both oils have, such as myristic acid, palmitic acid, oleic acid, phthalic acid,

**Table 2. The Compound Content Candlenut Oil and VCO**

Candlenut Oil	VCO
Lauric Acid	Caprylic Acid
Myristic Acid	Capric Acid
Palmitic Acid	Myristic Acid
Margaric Acid	Palmitic Acid
Stearic Acid	Oleic Acid
Oleic Acid	Phthalat Acid
Arachidic Acid	Arachidic Acid
Linoleic Acid	Lauric Acid
Behenic Acid	-
Phthalat Acid	-

arachidic acid and lauric acid. Boateng et al., (2016) stated that VCO contains caprylic acid (8%), capric acid (7%), lauric acid (49%), myristic acid (8%), palmitic acid (8%), stearic acid (2%), oleic acid (6%) and linoleic acid (2%) (Table 2). The analysis of VCO compound content in this study found similar compounds, specifically caprylic acid, capric acid, myristic acid, palmitic acid, oleic acid, and lauric acid. Candlenut oil was found to contain oleic acid and linoleic acid compounds, based on the statement by Yuliani et al., (2018), 91.77% of candlenut oil consists of oleic acid, linoleic acid and linolenic acid. From the GC-MS reading results, it can be seen that there are several different compound contents, the differences in compound content in the oil are influenced by several factors, one of which is the processing process of the oil itself (Ludya Pulung et al., 2016; Sun et al., 2022). The samples in this study have met the standards and are ready to be used as active ingredients for making nanoemulsions.

### Formulation of Nanoemulsion

#### Organoleptic test

The result of organoleptic test can be seen in Table 3. Based on the result of organoleptic test, Formula 1 was an unstable formula because after

several days of storage, there was separation in the nanoemulsion, while Formulas 2 and 3 remained stable. This shows that the ratio of VCO and candlenut oils influenced the stability of the nanoemulsion.

**Table 3. Result of Organoleptic Test**

Organoleptic	Formula 1	Formula 2	Formula 3
Color	yellow	yellow	yellow
odor	distinct odor	distinct odor	distinct odor
Viscosity	viscous	viscous	viscous
Separation	yes	no	no

#### pH test

The result of pH test can be seen in Table 4. Based on table 4, the pH of each nanoemulsion formula can be seen to be in the range of 7.05 - 7.14. These values meet the pH range specified by SNI-1996 of 4.5 to 8 and nanoemulsions with a pH in the range of 6.5-9.0 have good dispersibility and transparency without flocculation, coagulation, segregation and also show good stability (Son et al., 2019).

**Table 4. Result of pH Test**

Formula	Average of pH $\pm$ SD
1	7.05 $\pm$ 0.08
2	7.14 $\pm$ 0.120
3	7.12 $\pm$ 0.175

#### Transmittance test

Based on Table 5, the % transmittance of the three formulas approached 100%, indicating that the droplet size of the dispersion produced by the nanoemulsion had reached the nanometer size (Senapati et al., 2016). Formula 2 had results closest to 100%.

**Table 5. Result of % Transmittance Test**

Formula	Average of % Transmittance $\pm$ SD
1	93.02 $\pm$ 0.11
2	96.60 $\pm$ 0.26
3	96.38 $\pm$ 0.18

## Viscosity and Flow Type Test

Based on the **Table 6**, it can be known that the viscosity values of the three formulas were in the range of 200-550 cP, which falls within the viscosity range for nanoemulsions of 10-2000 cP. Formula 1 had a high viscosity value of  $548.873 \pm 11.91$  cP, which was very different from Formulas 2 and 3, likely due to the instability of Formula 1. The flow type of 3 formulas is Newtonian, according to Kong et al., (2018), the common flow type possessed by nanoemulsion formulations is Newtonian type due to the low viscosity of the formulations.

**Table 6. Result of Viscosity and Flow Type Test**

Formula	Average of viscosity test (cpa's) $\pm$ SD	Flow Type
1	$548.873 \pm 11.91$	Newtonian
2	$247.55 \pm 7.18$	Newtonian
3	$241.07 \pm 6.38$	Newtonian

Based on the results of organoleptic evaluation, pH, viscosity, flow types and % transmittance tests, formula 2 was chosen as the best formula. Formula 2 was then further characterized by continued testing of droplet size and zeta potential. Droplet size and zeta potential were tested using the Particle Size Analyzer (PSA).

**Table 7. Result of Droplet Size and Potential Zeta Test of Formula 2**

Droplet Size $\pm$ SD	PDI $\pm$ SD	Potential Zeta $\pm$ SD
$14.42 \pm 0.03$ nm	0.28	$-27.8 \pm 1.02$ mV

Based on **Table 7**, it can be seen that formula 2 has a droplet size of  $14.42 \pm 0.03$  nm which meets the droplet size range for nanoemulsions, namely  $< 100$  nm (Chavda et al., 2022). The polydispersion index value of 0.28 ( $< 0.3$ ) indicates that the preparation can be categorized into monodisperse and homogeneous, this is also supported by the statement of (Handayani et al., 2018) which states that the polydispersion index value in the range of 0.3 represents that the globules in the nanoemulsion preparation are homogeneously distributed. The potential zeta value obtained in formulation 2 is  $-27.8 \pm 1.02$  mV which doesn't meet the requirements stated

by (Mappamasing et al., 2015). The potential zeta value is expected to be between +30 and -30, this indicates that formula 2 nanoemulsion is unstable but Zeta potential is not the main parameter in determining the stability of a nanoparticle, some other factors that also influence stability include particle size, distribution and morphology (Shah et al., 2014).

## Hair Growth Activity Test

Treatment groups and hair growth measurement results can be seen in the **Table 8**. Based on the two-way ANOVA test, it was found that on day 7 there was no significant difference in hair length between all groups (significance  $< 0.05$ ). Differences in hair length began to appear on day 14 with the nanoemulsion group having a significantly longer hair length compared to other groups, indicating that the performance of nanoemulsions is faster than other groups, especially the VCO and conventional candlenut oil groups. This is in accordance with the purpose of making nanoemulsions, which is to increase the absorption of active ingredients so as to accelerate hair growth. On day 28, the nanoemulsion group showed significant difference in hair length with the negative group but did not show a significant difference with other treatment groups, which can be interpreted that all treatment groups showed good hair growth activity and were as good as nanoemulsions.

The nanoemulsion group had significant differences compared to the negative control group. This means that the base formula used in the nanoemulsion did not affect the rate of hair growth. However, what did influence the rate of hair growth was the combination of VCO and candlenut oil in a ratio of 1:3. It can be seen that the treatment of emulsion, positive control, candlenut oil, VCO and nanoemulsion had a hair growth effect, but the one that gave the highest hair growth was the VCO and nanoemulsion group. The VCO and nanoemulsion treatment groups did not show any significant differences, so it can be seen that the hair growth effect is influenced by the compounds contained in VCO.

Based on research conducted by Blegur & Indrawati (2015), they tested the hair growth activity of rabbits using 100% VCO emulsion. It was found that hair growth was 0.9 mm for 18



**Table 8. Measurement of Hair Growth Activity Each Week**

Group	Measurement of Hair Growth (mm) Activity Each Week			
	Day - 7	Day - 14	Day - 21	Day - 28
Emulsion	1.48 ± 0.25 <sup>a</sup>	2.12 ± 0.31 <sup>a</sup>	5.84 ± 0.40 <sup>b</sup>	15.92 ± 0.61 <sup>bc</sup>
Candlenut Oil	1.71 ± 0.09 <sup>a</sup>	2.78 ± 0.25 <sup>a</sup>	4.91 ± 0.53 <sup>a</sup>	16.30 ± 1.28 <sup>bc</sup>
VCO	1.76 ± 0.03 <sup>a</sup>	2.31 ± 0.18 <sup>a</sup>	7.44 ± 0.42 <sup>cd</sup>	17.58 ± 1.19 <sup>c</sup>
Negative Control (Nanoemulsion without active ingridients)	1.55 ± 0.58 <sup>a</sup>	2.42 ± 0.32 <sup>a</sup>	8.61 ± 0.78 <sup>d</sup>	9.12 ± 0.68 <sup>a</sup>
Positive Control (Ijot's Secret Hairoil®)	1.87 ± 0.43 <sup>a</sup>	2.54 ± 0.62 <sup>a</sup>	7.16 ± 0.63 <sup>c</sup>	16.58 ± 0.91 <sup>bc</sup>
Nanoemulsion (Formula 2)	2.04 ± 0.55 <sup>a</sup>	3.62 ± 0,71 <sup>b</sup>	10.32 ± 0.70 <sup>e</sup>	17.14 ± 0.42 <sup>c</sup>

Note: Different letters (a, b, c, d, e) for each formula indicate there are significant differences ( $p < 0.05$ )

days. Meanwhile, in this current study the hair length of the emulsion group that used a combination of VCO and hazelnut oil in a 1:3 ratio was  $15.92 \pm 0.61$  mm. This means that the combination of VCO and 1:3 candlenut oil had a higher hair growth effect compared to the 100% VCO emulsion group tested in the previous study.

The hair weight of each group was measured, with this test being carried out on the 28th day of treatment by shaving the hair of the mice that had grown during treatment and weighing it using an analytical balance, and the results of average hair weight of each group can be seen in Table 9.

The results of the one-way ANOVA test showed a significance value of  $< 0.05$ , indicating a meaningful difference between each treatment group. With a significant difference in hair weight, a post hoc Tukey's test was carried out. From the Tukey's test, it was found that the nanoemulsion group had a significantly heavier hair weight compared to all other group. However, the candlenut oil group vs the emulsion group did not show a significant difference in hair weight ( $> 0.05$ ). This can be interpreted that the administration of candlenut oil and emulsion combination has the same effect on hair volume but not on hair length.

The VCO group and conventional candlenut oil also showed significant differences in hair weight, the candlenut group showed higher hair weight compared to the VCO group but the VCO

group showed more good hair growth activity than the candlenut group so it can be interpreted that the administration of VCO can accelerate hair growth and candlenut oil can increase hair thickness.

**Table 9. Measurement of Hair Weight On 28th day**

Group	Hair Weight (mg)
Emulsion	372.77 ± 28.36 <sup>d</sup>
Candlenut Oil	367.49 ± 15.53 <sup>d</sup>
VCO	112.52 ± 24.76 <sup>a</sup>
Negative Control	175.68 ± 26.67 <sup>b</sup>
Positive Control	241.36 ± 22.55 <sup>c</sup>
Nanoemulsion	699.37 ± 7.91 <sup>d</sup>

Note: Different letters (a, b, c, d, e) for each formula indicate there are significant differences ( $p < 0.05$ )

Based on the results of the hair length test, it was found that the VCO group and nanoemulsion group had the highest hair length, but in the hair weight test there was a very significant difference between the VCO group and nanoemulsion group, so it can be interpreted that the treatment using a 1: 3 combination of VCO and candlenut oil nanoemulsion produces the best hair growth rate and hair weight. This is in accordance with research conducted by Almas et al., (2022) who conducted a hair growth activity test using 7% candlenut oil nanoemulsion in mice for 21 days and found hair

length of 3.649 mm and hair weight of 6.70 mg. Similar research was also conducted by Shoviantari et al., (2019) stating that rabbits given 5% candlenut oil nanoemulsion treatment had higher hair length and thickness compared to other groups.

From the results of the hair length test, it was found that the VCO group and the nanoemulsion group had the highest hair length, but in the hair weight test there was a very significant difference between the VCO and nanoemulsion groups, so it can be interpreted that the treatment using a nanoemulsion combination of VCO and candlenut oil 1:3 resulted in best hair growth and hair weight.

## CONCLUSIONS

The results of the study concluded that applying a nanoemulsion combination of VCO and candlenut oil in a ratio of 1: 3 gave a growth rate of  $17.14 \pm 0.42$  mm and hair weight of  $699.37 \pm 7.91$  mg. It is necessary to increase the variations of ratios to determine the potential of good oil ratios. Going forward, clinical trials can also be carried out regarding the use of this nanoemulsion to determine its hair growth activity.

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## AUTHORS' CONTRIBUTIONS

Lasanudin, Bachri, Wahyuningsih designed the study. Lasanudin performed the experiments. Bachri and Wahyuningsih helped supervise the project. Lasanudin and Wahyuningsih wrote the paper with input from all authors.

## CONFLICT OF INTERESTS

The authors have no conflict of interests related to this publication.

## ETHICAL CONSIDERATION

Ethical clearance for experimental animals has been registered with the ethics committee of The Ahmad Dahlan University (KEP UAD) with No.012311284

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