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Original Article

Examining the Impact of Candlenut Fruit Extract Cream on Hair Growth in Male Wistar Rats (Rattus norvegicus)

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ABSTRACT

Hair loss can affect mental health. Natural compound-based hair treatments like candlenut fruit are becoming more popular due to their low side effects and growing scientific research on hair loss. This study aims to prove the effectiveness of candlenut fruit extract cream on the hair growth of male Wistar strain rats (Rattus norvegicus). Research method Laboratory experiment, Principle 3R (Replacement, Reduction, and Refinement): Reducing Rattus norvegicus Wistar male strain samples The 20 male rats in this study will be divided into four groups. Hair growth is examined with 5%, 10%, and 15% candlenut fruit extract cream. Research data was analyzed using SPSS. The results of testing hair growth in rats with 5%, 10%, and 15% doses of candlenut fruit extract cream were normally distributed. The control group had a significant value of 0.928 > 0.05, treatment group 1 had a value of 0.579 > 0.05, treatment group 2 of 0.829 > 0.05, and treatment group 3 of 0.451 > 0.05. Candlenut fruit extract cream is effective for increasing hair growth in Rattus norvegicus. This study concludes that candlenut fruit extract cream can accelerate the development of hair follicles in Wistar strain male white rats. Due to the heated manufacturing process, candlenut extract is safe to use.

Keywords: *Candlenut, Hair Loss, Follicles, Hair Health*

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INTRODUCTION

Men and women alike care a lot about the hair on their bodies. Taking care of your hair is vital to look good and stay healthy, especially for the head. Many people are willing to spend much money to care for their hair or make it grow thick and full.

Hair health can affect personal distress and psychological well-being, especially when causing hair loss [1]. Treatment options include pharmacological and cosmetic procedures, addressing genetics, diseases, medications, lifestyle, chemical exposure, and unhealthy habits to promote growth and health [2].

Hair loss has a significant social, psychological, and emotional impact, necessitating treatment and improved health. Humans have approximately 100,000 hair shafts with varying growth rates, with the medulla, cortex, and cuticle layers [3–7]. Hair follicles can be divided into lower, middle, and upper segments, with the lower segment encompassing bulbous and supra bulb regions, the middle segment covering the arrector pili muscle, and the upper segment encompassing the follicular orifice [6]. The hair growth cycle has three phases: anagen (2–7 years), catagen (2–4 weeks), and telogen (3 months). Anagen (85–90.6%), telogen (10–15%), and catagen (1–2%) phases affect scalp hair [8,9].

Hair loss affects 50 million people in the US, with androgenetic alopecia being the most common, particularly in men. Female pattern hair loss (FPHL) is prevalent in over 50% of women over 79. Alopecia areata and

moth heads are also common [10]. Global epidemiological data on hair loss prevalence in Indonesia is unavailable. The Skin and Venereology Polyclinic at Cipto Mangunkusumo Hospital studied 116 patients with hair loss and found that alopecia areata (39.7%), telogen effluvium (34.5%), and androgenic alopecia were the most common types—(11.2%) [11].

Natural compound-based treatments for hair problems are becoming popular due to side effects and increasing scientific research on hair loss. Candlenut fruit, a natural ingredient with high levels of omega fatty acids, is beneficial for hair health and can be used as a food source. However, its application in food products needs to be increased by encapsulation techniques because its odor and thermal sensitivity are susceptible to oxidation [12,13].

Previous research used encapsulation material, oil ratio, and moisture content to make microcapsules from candlenut oil [14,15]. The highest efficiency was achieved with sodium caseinate at 43.22 ± 0.9%, and the second stage increased the efficiency to 64.86% with sodium caseinate [16]. In addition, another study found that candlenut oil, also known as *Aleurit Moluccana L. Willd*, is effective as a hair grower. They used Nanostructured Lipid Carrier (NLC) and cocoa beeswax-oleum to obtain better NLC characteristics. So, it has more excellent hair growth activity [17].

However, few studies have shown how to use candlenut preparations other than oil for hair growth. Candlenut's hair growth chemicals have yet to be fully explained. Thus, health science research on candlenut fruit benefits, especially natural ingredient research, must be expanded.

METHOD

Laboratory experiments (true experimental) were used to test candlenut (*Aleurit Moluccana*) fruit extract cream on hair growth of male rats (*Rattus norvegicus*) Wistar strain using a post-test with control group design or control group with treatment [18,19].

Male mice were chosen because they do not have estrogen, or if they have, there are few of them, and their hormonal state is more consistent than female mice's during estrus, lactation, and pregnancy. Because of their quick metabolism, Wistar rats are better for body metabolism research[20].

Using the 3R Principle (replacement, reduction, and refinement), researchers reduced the number of research samples [21]. A total of 20 male rats in this study will be divided into four groups: The control group (K), consisting of 5 male rats, was not given candlenut fruit extract cream. The second group (P1) consisted of 5 male rats that were given 5% candlenut fruit extract cream topically. The third group (P2) consisted of 5 male rats given 10% candlenut fruit extract cream topically. The fourth group (P3) consisted of 5 male rats that received 15% candlenut fruit extract cream topically.

Variables are characteristics or attributes that can be measured or observed and vary among people or organizations. This study observed 5%, 10%, and 15% candlenut fruit extract cream variables as independent variables, and the dependent variable was hair growth.

Rat cages, gloves, knives, digital scales, markers, blenders, pipettes, 3ml syringes, feed containers, mixers, freezers, freeze dryers, masks, microplate readers, microscopes, staining jar, coated desk glass, cover glass, and laptops were among the research tools utilized in this study. Label paper, pure water, 96% ethanol, 1% HCL, physically unimpaired male rats, rat food and drink, candlenut fruit extract, and other research supplies were utilized.

Acclimatization of experimental animals, preparation of candlenut fruit extract cream, phytochemical test, antioxidant test, and treatment were part of the research process.

Acclimatization—adjusting to a new location, climate, circumstances, or atmosphere—begins the research process. All male mice, young and elderly, will acclimatize for seven days at MEDA, Faculty of Mathematics and Natural Sciences, University of North Sumatra, before treatment. Rats are given time to adjust to their new environment, food, and drink. Food and drink are provided ad libitum to rats.

Fruit and seeds from candlenut bushes will make Candlenut Fruit Extract Cream. Candlenut seeds and fruit are dried at 50-60°C and crushed into powder. Making candlenut fruit extract requires maceration. We extracted dried candlenut fruit powder with 96% ethanol, filtered it, and then macerated the residue again. This solvent was chosen because ethanol can filter polar, semipolar, and nonpolar active substances. We hope it produces the most

extract. A rotating evaporator evaporates ethanol to make a thick extract. Evaporation results are water-bathed to thicken. The cream is created from the thick extract. Cream preparation requires melting and emulsifying. Oil and wax are melted in a water bath at 70–75°C, while heat-resistant aqueous solutions and water-soluble components are heated at the same temperature as fat. After adding the aqueous solution to the liquid fat mixture and stirring constantly, the temperature is maintained for 5-10 minutes to prevent wax/fat crystallization. Next, the mixture is slowly chilled while stirring until thickened. If the aqueous solution is not at the same temperature as the melted fat, some wax will solidify, separating the fat and liquid phases. The completed cream is placed in a container or tube like the ointment. Cream preparations can be destroyed if the mixing mechanism is disturbed, mainly due to temperature fluctuations and composition changes from the excessive addition of one phase or two creams without mixing the emulsifying agents. Cream dilution requires a suitable diluent. Diluted cream should be used within a month.

Then, a phytochemical test is carried out as an initial testing method to determine the active compound content contained in the plant so that it can be used as medicine.

Then, DPPH antioxidant testing was done. This method uses DPPH's drastic color change. Yellow 1,1-diphenyl-2-picrylhydrazine is formed when the DPPH free radical reacts with one hydrogen atom from the test substance.

The formula calculated antioxidant activity (% IC):

$$\% \text{ IC} = \frac{\text{Abs Blank} - \text{Abs Sample}}{\text{Abs Blank}} \text{ x } 100\%$$

The Antioxidant Activity Test Method is validated by calculating the precision value (% CV) of specificity and linearity with the formula: Standard deviation of the measured concentration divided by the average theoretical concentration multiplied by 100%.

Antioxidant data on ABTS radicals (% inhibition) of candlenut fruit ethanol extract was examined, and the IC50 value was obtained. Smaller IC_{50} values indicate higher antioxidant action. The IC_{50} value was derived using linear regression in this investigation.

The IC_{50} value is calculated using resistance and solution concentration data using the linear regression equation y = a+ bx,

Where y is the % resistance 50 (value 50) and x is the IC_{50} value.

The levels of antioxidant activity can be described as follows: Powerful (IC_{50} < 50 µg/ml), Strong (IC_{50} 50-100 µg/ml), Moderate (IC_{50} 101-150 µg/ml), and Weak (IC_{50} > 150 µg/ml).

Topical lidocaine anesthetic is used to treat the back. Arrange the sedated rat prone on the surgical table. Mice backs were disinfected with 10% povidone iodine. I was shaving the disinfected rat's back. Topical 5% hazelnut fruit extract cream was applied daily to Group 1 mice for 21 days. Topical 10% hazelnut extract cream was applied daily to group 2 mice for 21 days. The 15% hazelnut extract cream was applied daily to group 3 mice for 14 days. The control group (K) received no therapy. Hair growth was measured every three days for up to 14 days in each mouse group. Tabulate and evaluate the data.

So, SPSS was used to examine every group's mouse hair development data. The paired T-test was continued after the normalcy test using Shapiro-Wilk was applied due to the small sample size (less than 50).

RESULTS

The rats used in tests are male *Rattus norvegicus* male Wistar strain rats weighing 150–600 grams and measuring 18–25 cm [22]. The candlenut extract cream has different percentages for each group. Apply lidocaine liquid to the back for anesthesia. After placing the anesthetized rat prone on the surgical table, povidone-iodine 10% disinfected its back. The next step was shaving rats with a 4x4 cm hair shaver—each group's candlenut fruit extract cream treatment.

Cream preparation involves melting and emulsifying. Melting oils and waxes in a water bath at 70-75°C is typical. Choose, wash, heat, and mash high-quality candlenuts until the next step. In all heat-resistant aqueous solutions, the water-soluble component is heated at the same temperature as the fat component. Using constant stirring, the mixture is slowly cooled and thickened. If the aqueous solution is not at the same temperature as the fat fusion, some waxes will solidify, separating the fat and liquid phases. The finished cream is packaged in a bottle or tube like the ointment.

The candlenut fruit extract alkaloid study showed positive results in Table 1. The extract was tested with Meyer, Dragendorf, and Wanger preaction reagents. Red-brown flavonoids were found. The extract tested negative for saponin, tannins, and terpenoids/steroids. Steroids and tannins did not change color.

DPPH-based antioxidant activity testing followed. This uses DPPH radical color change. A yellow 1,1-diphenyl-2-picrylhydrazine compound is formed when the DPPH free radical reacts with one hydrogen atom from the test material. DPPH is fast and thorough, making it simple. After weighing 0.0504 mg of candlenut (*Aleurit Moluccana*), oil dissolved in 96% ethanol, a 50.0 ml flask is filled to 1000 ppm. At 500 ppm ethanol, 25.0 ml pipetted into a 50.0 ml flask is enough. Pipetted samples were 1.0, 2.0, 4.0, and 8.0 ml. In a 20.0 ml flask, add enough ethanol to the mark line to get 25 ppm, 50 ppm, 100 ppm, and 200 ppm concentrations.

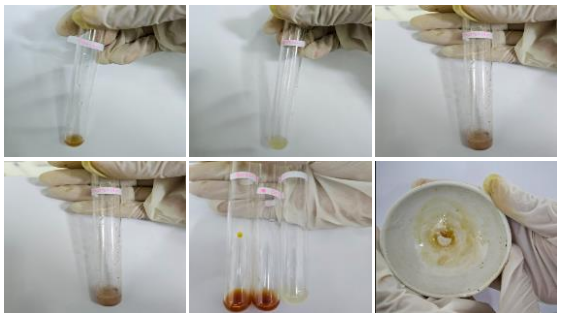

Figure 1. Phytochemical Screening Result of Candlenut Extract.

Weighting 10 mg of DPPH powder in a flask of ethanol yielded 40 ppm. After homogenizing the blank sample, 400 - 800 nm absorbance was measured. For 60 minutes, DPPH concentration in methanol was measured.

Table 1. Candlenut Extract Test (ppm)

Sample Name	IC_{50} Rep. 1	IC_{50} Rep. 2	IC_{50} Rep. 3
Candlenut extract	157,5728	160,8232	160,7409

IC_{50} values at 516 nm are 157.5, 160.8, and 160.7 at minutes 0, 30, and 60. The antioxidant in candlenut oil is weak because Vitamin E has an IC_{50} of 68 ppm at minute 0, 41 ppm at minute 30, and 45 ppm at minute 60. Pure Vitamin E has higher antioxidant activity than candlenut oil because no other compounds can interfere with radical silencing. Thus, candlenut oil's antioxidant is weak at 151-200 ppm. Antioxidants donate electrons to fight free radicals.

The primary test is rat hair growth after different treatments. The control group received no treatment. Treatment group 1 received 5% candlenut fruit extract cream topically daily for 21 days. Group 2 Treatment Treatment Group 2 rats received 10% candlenut fruit extract cream topically daily for 14 days. Treatment group 3 rats received 15% candlenut fruit extract cream topically daily for 14 days.

All groups show hair growth, but treatment group 1 has the fastest hair growth with 1.66 cm for the 4th rat sample. Researchers calculated each treatment group's average hair growth and standard deviation to determine the quickest growth.

Table 2. Average Observation Results of Hair Length (cm)

Group	Repetition	D3	D6	D9	D12	D15	D18	D21	Average
Control (P0)	1	0	0	0,24	0,33	0,46	0,52	0,61	0.31
	2	0	0	0,21	0,3	0,43	0,5	0,59	0.29
	3	0	0	0,19	0,28	0,39	0,48	0,57	0.27
	4	0	0	0,25	0,34	0,47	0,56	0,62	0.32
	5	0	0	0,2	0,32	0,44	0,54	0,63	0.30
Treatment 1 (P1)	1	0,37	0,68	0,76	0,84	0,95	1,51	1.62	0.85
	2	0,35	0,65	0,74	0,84	0,97	1,55	1,63	0.96
	3	0,28	0,63	0,74	0,83	0,95	1,54	1,6	0.94
	4	0,38	0,71	0,82	0,91	1,08	1,58	1,66	1.02
	5	0,36	0,69	0,8	0,9	1,09	1,55	1,64	1
Treatment 2 (P2)	1	0,42	0,78	0,88	0,98	1,12	1,33	1,52	1

	2	0,41	0,74	0,87	1,04	1,18	1,35	1,56	1.02
	3	0,39	0,73	0,85	0,98	1,09	1,25	1,48	0.97
	4	0,44	0,82	0,93	1,09	1,21	1,39	1,58	1.07
	5	0,46	0,83	0,99	1,12	1,25	1,41	1,63	1.1
Treatment 3 (P3)	1	0,38	0,41	0,56	0,71	0,88	0,97	1,18	0.73
	2	0,42	0,48	0,62	0,81	0,92	1,16	1,25	0.81
	3	0,28	0,34	0,55	0,7	0,94	1,12	1,23	0.74
	4	0,35	0,4	0,57	0,7	0,89	1,1	1,18	0.74
	5	0,27	0,39	0,41	0,59	0,72	0,96	1,11	0.64

Note: Composition Kemiri fruit extract cream. The control group (P0) was not given, Treatment group I (P1) + 5% level topically, Treatment group II (P2) + 10% level topically and Treatment group III (P3) + 15% level topically.

Table 3. Paired T-Test Results

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Group Result	1.64250	.86163	.19267	1.23924	2.04576	8.525	19	.000

Note: Sig. (2-tailed) < 0.05 reject H0, Sig. (2-tailed) > 0.05 accepted H0

The average hair follicle growth in rats with 5%, 10%, and 15% candlenut cream extract shows significant results with no treatment (Control Group). The control group, treatment 1, treatment 2, and treatment three were observed for 21 days. The average cream extract application in treatment group 2 produced the most extended hair growth, 1.1 cm. Thus, candlenut fruit cream extract accelerates hair follicle growth in male Wistar rats (*Rattus norvegicus*).

Table 4. Shapiro Wilk Normality Test.

Group		Shapiro-Wilk		
		Statistic	df	Sig.
Result	P0	.979	5	.928
	P1	.927	5	.578
	P2	.963	5	.829
	P3	.907	5	.451

If the number of samples is more than 50, then the normality test used is Kolmogorov-Smirnov, whereas if the sample is less than 50, then the Spahiro-Wilk test is used. Because this study had less than 50 samples, the Shapiro-Wilk test was used, with the significance level in the normality test being > 0.05. The data normality test was analyzed using the Kolmogorov-Smirnov test approach (p > 0.05) [23]. From the data in Table 4, it can be seen that the results of the normality test using Shapiro Wilk show that the significant value of the control group is 0.928 > 0.05, so the data is normally distributed, the significant value of treatment group 1 is 0.579 > 0.05, so the data is normally distributed, for the considerable value of treatment group 2 of 0.829 > 0.05, the data is normally distributed. The significant value of treatment group 3 is 0.451 > 0.05, so the data is normally distributed.

When the population diversity is uncertain, the data does not follow a normal distribution, or there is a small sample size (n), specifically less than 30, the t-test is employed. The data is considered to be near normal if the data is greater than 30 and the population variation is unknown, according to the Central Limit Theorem (CLT). Statistical significance (Sig.) from SPSS-generated findings informed a paired sample t-test. H0 is rejected if the Sig. (2-tailed) value is less than 0.05, and H0 is accepted if the Sig. (2-tailed) value is more than 0.05 [23].

This paired t-test aims to compare and evaluate data from two different variables. On days 3 and 21, researchers compared the treatment groups that had received 5%, 10%, or 15% hazelnut cream extract with those that had not. The sig value is visible in Table 3 of the results of the paired samples test mentioned earlier. A significant difference in hair growth between groups can be inferred from the fact

that the 2-tailed value is 0.00 < 0.05. The sig value can be seen from the paired samples test output table data above. (2 tailed) is 0.00 <0.05, so there is a significant difference in hair growth between groups.

DISCUSSION

An optimal condition of the scalp is essential for maintaining healthy hair; conversely, the hair's health contributes to the overall well-being of the scalp. Alopecia can be caused by inadequate hair and scalp condition. Alopecia affects individuals of all genders and age groups [24]. Hair loss can be attributed to various factors, such as environmental factors (radiation and chemicals), hormonal imbalances, nutritional deficiencies, and Genetic predisposition [25,26]. A simple method to prevent hair loss and stimulate follicle growth is to enhance blood circulation to the scalp and massage it using hair. Certain creams can alleviate hair conditions. Hair creams provide essential nutrients to the hair follicles, supporting optimal hair growth.

Solvent and extraction procedures affect phytochemical screening. Plant secondary metabolites include alkaloids, flavonoids, steroids, saponins, terpenoids, and tannins. The treatment group received candlenut fruit extract at the prescribed dose to test for active components affecting mouse hair development. The candlenut fruit extract tested positive for alkaloids. This alkaloid test involved introducing 0.5 grams of extract to a test tube, adding chloroform and ammonia, and then testing with Meyer, Dregendof, and Wagner reactions. The positive solution in Meyer's reagent is due to a white residue, while Dragendorf's is due to the solution turning orange-red and then brown.

Flavonoid Analysis A test tube contained 0.5 grams of extract, ethanol, and concentrated HCI. Mg powder (0.2 grams) was added to the solution. A red-brown color indicates flavonoids. This study has flavonoids. Saponin Test: Add 0.5 gram of extract to 10 mL of distilled water and mix vigorously for 1 minute. Stable foam for 10 minutes indicates saponin chemicals in the sample. Candlenut extract lacks saponin, according to the findings. Tannin and terpenoid/steroid tests provide negative results since the solution does not turn blue or green. Or black. Candlenuts exclusively contain alkaloids and flavonoids, according to phytochemical tests.

Because of their bactericidal activity, flavonoids can destroy the germs that cause hair and scalp loss and speed up the process of hair development [27,28]. Baldness and hair loss can occur when bacteria on the scalp and hair clog the hair follicles, a condition known as folliculitis. Flavonoids work by destroying the cell walls of bacteria, which in turn promotes hair growth. Damage to bacterial cell walls can be caused by an alcohol group in flavonoid compounds [29]. Hence, flavonoid chemicals can limit bacterial growth by entering the nucleus of bacterial cells. The prevention of baldness, acceleration of hair development, and strengthening of the capillary walls in the blood vessels of hair follicles can be achieved by inhibiting the proliferation of these bacteria.

Hair creams are costly, whereas candlenuts are affordable and advantageous. Candlenut oil extract typically stimulates the growth of hair follicles [30,31]. Nevertheless, the researchers employed candlenut cream to reassess its efficacy in promoting hair follicle growth in the Wistar strain white rats (*Rattus norvegicus*) [32]. To remove toxic toxalbumin, the cream was heated. The ointment is heavier and denser than the cream. The non-greasy cream has improved skin absorption kinetics and is easy to apply and remove. Ointments are less preferred than creams. Candlenut oil has low antioxidant activity at 151-200 ppm using the DPPH method.

Candlenut fruit extract was administered to Wistar strain white rats (*Rattus norvegicus*) to see if it is effective in growing hair. This study used male Wistar strain white rats (Rattus norvegicus). Using the 3R Principle (Replacement, Reduction, and Refinement), the researchers reduced the number of study samples [21]. Four groups of test animals received treatment. The first group received 5%, the second 10%, and the third 15% candlenut fruit extract cream. The control group received no treatment.

After 21 days of observation, the data were tested using Shapiro-Wilk and paired with a T-test. The significant value of the control group was 0.928 > 0.05, so the data was normally distributed. The significant value of treatment group 1 was 0.579 > 0.05, the significant value of treatment group 2 was 0.829 > 0.05, and the significant value of treatment group 3 was 0.451 > 0.05.

The paired T-test tests normally

distributed data. We measured the effectiveness of the treatment by comparing the means before and after treatment. Paired T-test with sig value. This study found significant differences in hair follicle growth on day 21 between the control group, treatment 1 with 5% candlenut extract, treatment 2 with 10%, and treatment 3 with 15% candlenut extract (2-tailed p-value < 0.05) so that the administration of candlenut cream is effective for the growth of white rat hair follicles (*Rattus norvegicus*) Wistar strain.

CONCLUSION

The research procedure was carried out for 21 days of observation and produced data that needed to be tested first using Shapiro Wilk and continued with paired T-test. The normality test results using Shapiro Wilk showed the significant value of the control group, treatment 1, treatment 2, and treatment 3 > 0.05, so the data was normally distributed.

The paired samples test above can be seen in the sig value. (2 tailed) is 0.00 <0.05, so there is a significant difference in hair growth between groups. So, it can be concluded that candlenut (*Aleurit Moluccana L. Willd*) fruit cream extract can accelerate hair follicle growth in male Wistar strain rats (*Rattus norvegicus*). Candlenut extract is also safe because the manufacturing process is first heated to remove the toxins in the candlenut fruit.

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