THE EFFECT OF COMPARISON OF CANDLECRON OIL COMBINATIONSAND VCO IN HAIR TONIC FORMULA ON HAIR GROWTH IN WHITE RATS (*Rattus norvegicus*)

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ABSTRACT

This study aimed to evaluate the effects of different combinations of candlenut oil and virgin coconut oil (VCO) in hair tonic formulations on hair growth in white rats (Rattus norvegicus). A true experimental study using a Completely Randomized Design was conducted with three formulations: Formula I (5 mL candlenut oil and 5 mL VCO), Formula II (5 mL candlenut oil and 10 mL VCO), and Formula III (10 mL candlenut oil and 5 mL VCO). Hair growth was observed over 28 days, with negative and positive controls included for comparison. The formulations were evaluated through organoleptic, hedonic, homogeneity, pH, and viscosity tests. All three formulations were homogeneous, had a pH of 5, and exhibited a viscosity of 12 cP. Based on the reported descriptive results, Formula I showed the highest mean hair growth among the experimental formulations (7.64), followed by Formula II (5.08) and Formula III (4.22). The positive and negative controls recorded mean values of 8.89 and 2.77, respectively. However, statistical analysis revealed no significant difference among the groups ($p = 0.406$). Therefore, the apparent advantage of Formula I was descriptive and did not establish statistically superior efficacy. The findings provide preliminary information on the formulation characteristics and potential of candlenut oil and VCO combinations for hair tonic development. Further research should verify the original data, standardize the total oil concentration, and use adequately powered experiments to establish their effects on hair growth.

Keywords: *candlenut oil, virgin coconut oil, hair tonic, hair growth, Rattus norvegicus*

INTRODUCTION

Hair growth is a complex physiological process regulated by the hair follicle cycle, which consists of three primary phases: anagen (growth), catagen (regression), and telogen (resting). The duration and progression of these phases determine hair length, density, and overall follicular activity. Hair growth can be influenced by various biological and environmental factors, including hormonal regulation, oxidative stress, inflammation, and the condition of the surrounding skin. Consequently, the development of topical formulations containing biologically active natural ingredients has attracted interest in cosmetic and pharmaceutical research. Natural oils are particularly relevant because their fatty acid composition and other bioactive constituents may provide beneficial properties for skin care and the maintenance of a healthy environment for hair follicles.

Virgin coconut oil (VCO) is a naturally derived oil obtained from fresh coconut kernels and is characterized by its

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high content of medium-chain fatty acids, particularly lauric acid. Its physicochemical properties and potential applications in pharmaceutical and cosmetic formulations have been discussed in previous research. Derawi et al. [1], investigated the enzymatic glycerolysis of VCO to produce monoacylglycerols and diacylglycerols, demonstrating the relevance of VCO as a lipid substrate for developing functional ingredients. In addition, Rozani et al. [2], reviewed VCO-based emulsions and their potential benefits, highlighting the importance of formulation strategies in determining the performance and applicability of VCO-containing products. These findings provide a basis for considering VCO as an ingredient in topical preparations, including hair tonic formulations, although its suitability must also be evaluated in relation to the intended biological outcome.

The potential biological benefits of VCO have also been investigated in experimental and antimicrobial studies. Nair et al. [3], examined VCO supplementation in mice exposed to cyclophosphamide-induced systemic toxicity, contributing to the broader investigation of its potential protective effects under experimental conditions. Meanwhile, Gayatri et al. [4], evaluated the antibacterial effects of VCO against chromogenic bacteria associated with dental black staining in children. Although these studies address outcomes other than hair growth, they illustrate the diversity of biological applications investigated for VCO. Nevertheless, evidence of antioxidant, protective, or antimicrobial activity should not automatically be interpreted as evidence of direct hair growth stimulation. The relationship between these properties and hair follicle activity requires specific experimental investigation.

Research into VCO has also explored its preparation, formulation behavior, and broader therapeutic potential. Rahmawati et al. [5], investigated the use of protease from palado (*Agave*) roots and leaves in the production of VCO, illustrating the relevance of extraction and processing methods to the development of coconut oil products. Gani and Adisah [6], examined the phase behavior of swiftlet nest preparations involving VCO and nonionic surfactants, providing a formulation-related perspective on systems containing this oil. Furthermore, Nasution et al. [7], mapped scientific publications on the therapeutic potential of VCO from 2004 to 2024 through bibliometric analysis. Their work reflects the expanding research interest in VCO and its possible applications. However, bibliometric evidence of growing scientific interest does not establish the efficacy of VCO for a specific indication, such as stimulating hair growth.

In addition to VCO, Candlecron oil is of interest as a potential ingredient in a hair tonic formulation. However, the available references do not provide sufficient evidence to establish its chemical composition, pharmacological properties, safety profile, or mechanism of action in hair follicles. Its contribution to hair growth therefore requires direct experimental evaluation rather than assumptions based on the general properties of natural oils. Comparing different combinations of Candlecron oil and VCO may help determine whether variations in their proportions are associated with differences in hair growth outcomes. Such an investigation is particularly relevant because the biological performance of a topical formulation may depend not only on

its individual ingredients but also on their relative concentrations and interactions within the formulation.

Despite the growing body of research concerning VCO, the references reviewed here primarily address its production, formulation characteristics, antimicrobial effects, potential protective activities, and broader therapeutic applications. They do not establish the comparative effectiveness of Candlecron oil and VCO combinations in hair tonic formulations for promoting hair growth. Moreover, the available evidence does not establish whether combining these oils produces greater hair growth than using individual ingredients or alternative combinations

White rats (*Rattus norvegicus*) provide an experimental model for investigating biological responses to topical formulations under controlled conditions. By applying hair tonic formulations containing different combinations of Candlecron oil and VCO, researchers can compare hair growth outcomes across treatment groups. Such an approach may provide preliminary experimental evidence regarding the relative performance of the formulations and support further investigations into their mechanisms, safety, and potential cosmetic applications. Nevertheless, findings obtained from animal models should be interpreted cautiously and cannot be directly generalized to human hair growth without additional evidence.

Therefore, this study aims to compare the effects of different Candlecron oil and VCO combinations in hair tonic formulations on hair growth in white rats (*Rattus norvegicus*). Specifically, the study seeks to determine whether differences in the oil combinations are associated with differences in hair growth outcomes. By addressing the limited experimental evidence concerning these combinations, the study is expected to contribute to the scientific evaluation of natural-oil-based hair tonic formulations and provide a foundation for subsequent research on their efficacy, safety, and potential application in hair care products.

Literature Review

Hair Growth Mechanisms

Hair growth is a dynamic biological process regulated by the hair follicle cycle, which consists of anagen (active growth), catagen (regression), and telogen (resting) phases. The transition between these phases is influenced by cellular activity, hormonal regulation, local signaling pathways, and the physiological condition of the skin. Oxidative stress may also affect cellular integrity and tissue homeostasis, making antioxidant activity a relevant research consideration in studies of hair and skin health. Illam et al. [8], demonstrated that polyphenols derived from virgin coconut oil (VCO) can protect against pro-oxidant-mediated cell death, suggesting potential biological relevance for oxidative stress management. However, this evidence does not establish a direct effect on hair follicle cycling or hair growth. Therefore, the relationship between antioxidant properties and hair growth stimulation requires further investigation using specific experimental outcomes.

Properties and Biological Activities of Virgin Coconut Oil

Virgin coconut oil (VCO) is a natural lipid obtained from coconut kernels and contains predominantly saturated fatty acids, particularly lauric acid, alongside minor bioactive constituents whose concentrations may vary according to extraction and processing methods. Rohman et al. [9], reviewed the extraction methods, physicochemical properties,

biological activities, and authentication of VCO, emphasizing the importance of its composition and production characteristics in determining product quality. Ghani et al. [10], compared the physicochemical properties and antioxidant capacities of VCO produced through wet and dry processing, demonstrating the relevance of processing methods to its chemical characteristics. Furthermore, Illam et al. [8], reported protective effects associated with VCO polyphenols in a pro-oxidant cell model. These findings support investigating VCO as an ingredient in topical formulations, although its potential antioxidant and emollient properties should not be interpreted as proof of direct hair growth stimulation.

Antioxidant, Anti-Inflammatory, and Skin-Protective Properties of VCO

The antioxidant and skin-protective properties of VCO have been investigated through several experimental approaches. Varma et al. [11], examined the in vitro anti-inflammatory and skin-protective properties of VCO, providing evidence relevant to its potential application in skin care. Zainodin et al. [12], investigated the antioxidant properties of VCO and its cytotoxicity toward human keratinocytes, addressing both potential biological benefits and cellular safety considerations. Similarly, Illam et al. [8], studied the effects of VCO and its polyphenol components against sodium fluoride toxicity using in vitro and in vivo models. Collectively, these studies indicate that VCO may have biological activities relevant to skin health. Nevertheless, evidence derived from inflammatory, oxidative-stress, or cytotoxicity models cannot independently establish whether VCO promotes hair follicle activity, and its effects on hair growth must be assessed directly.

VCO Formulation and Topical Applications

The formulation characteristics of VCO are important when considering its incorporation into hair tonic preparations because the physical stability, distribution, and delivery of lipid ingredients may influence product performance. Gani and Adisah [6], investigated the phase behavior of swiftlet nest preparations involving VCO and nonionic surfactants, providing insights into the interactions that may occur in multicomponent formulations. Noor et al. [13], explored empty nano- and microstructured lipid carriers involving VCO for skin moisturization, demonstrating the relevance of lipid-based delivery systems to topical applications. In addition, Annisa et al. [14] examined the characteristics of VCO microencapsulation using oil-in-water emulsification and complex coacervation methods. These studies establish that VCO can be investigated through different formulation approaches; however, they do not directly demonstrate the effectiveness of VCO-containing hair tonics or identify an optimal formulation for hair growth.

Experimental Studies on VCO in Animal Models

Experimental studies have investigated VCO in animal models to evaluate its biological effects under controlled conditions. Hima et al. [15], examined the effects of dietary VCO supplementation on immunity mediated through survival signaling pathways in rats, contributing to the understanding of its potential biological activity in vivo. Abujazia et al. [16], investigated the effects of VCO on bone oxidative status in ovariectomized rats, while Rahim et al. [17], examined memory enhancement in Wistar rats in association with antioxidative and cholinergic activities and reduced

oxidative stress. Nair et al. [3], also studied VCO supplementation in mice experiencing cyclophosphamide-induced systemic toxicity. Although these studies demonstrate that VCO has been investigated in different animal models and physiological contexts, their outcomes do not establish its effectiveness in promoting hair growth. Consequently, experiments using appropriate hair growth measurements are necessary to determine whether topical VCO formulations produce measurable effects on hair growth in *Rattus norvegicus*.

RESEARCH METHODS

Research Design

This study employed a true experimental research design using a Completely Randomized Design (CRD) to evaluate the effects of different combinations of candlenut oil and virgin coconut oil (VCO) in hair tonic formulations on hair growth. The experiment consisted of three formulations, designated Formula I, Formula II, and Formula III, which differed in the proportions of candlenut oil and VCO. The independent variable was the composition of the two oils, while hair growth measurements served as the dependent variable. Other formulation ingredients were maintained consistently across the three treatments to facilitate comparison. The study was conducted from August to September 2025, followed by approximately one month of data processing.

Research Tools and Materials

The equipment used in this study included dropper pipettes, vernier calipers, cotton buds, a shaving device, spatulas, measuring cups, a mixer, medium-sized containers, jars, filters or funnels, spoons, and tweezers. The materials consisted of candlenut oil, virgin coconut oil (VCO), 96% ethanol, methylparaben, menthol, polyethylene glycol 400 (PEG 400), propylene glycol, Tween 80, distilled water (aquadest), depilatory cream, cotton, and tissues. These materials were used to prepare the hair tonic formulations and support the experimental procedures for evaluating hair growth. The tools and materials were prepared before formulation and testing to ensure consistency throughout the experiment.

Hair Tonic Formulation

Three hair tonic formulations were prepared by varying the proportions of candlenut oil and VCO while maintaining the other ingredients at the same concentrations, as presented in Table 1. Each formulation contained 30% 96% ethanol, 0.3% methylparaben, 0.5% menthol, 5% PEG 400, 10% propylene glycol, and 1.5% Tween 80. Formula I contained 5 mL of candlenut oil and 5 mL of VCO, Formula II contained 5 mL of candlenut oil and 10 mL of VCO, and Formula III contained 10 mL of candlenut oil and 5 mL of VCO. Distilled water was added to bring each formulation to a final volume of 100 mL. The resulting formulations were intended to compare the effects of different oil combinations on hair growth under experimental conditions.

Table 1. Hair Tonic Formulations

Ingredients	Formula I	Formula II	Formula III
96% ethanol	30%	30%	30%
Methylparaben	0.30%	0.30%	0.30%
Menthol	0.50%	0.50%	0.50%
PEG 400	5%	5%	5%
Propylene glycol	10%	10%	10%
Tween 80	1.50%	1.50%	1.50%
Candlenut oil	5 mL	5 mL	10 mL
Virgin coconut oil (VCO)	5 mL	10 mL	5 mL

Distilled water	Add to 100 mL	Add to 100 mL	Add to 100 mL

Source: Primary data (2025).

Experimental Procedure and Hair Growth Assessment

The hair tonic formulations were prepared according to the specified ingredient proportions and final volume. The experimental procedure involved preparing the test area, applying the assigned formulation, and measuring hair growth using vernier calipers. Depilatory cream and a shaving device were listed among the materials and tools used for preparing the test area. Hair growth measurements were intended to provide a basis for comparing the outcomes associated with the three formulations. However, the available methodological information does not specify the animal allocation, number of animals per group, control group, application frequency, treatment duration, measurement intervals, or the precise method used to calculate hair growth. These details should be reported from the actual experimental records to ensure reproducibility and appropriate interpretation of the findings.

Data Analysis

The data were to be analyzed by comparing hair growth measurements among Formula I, Formula II, and Formula III. Because the study employed a Completely Randomized Design, a one-way analysis of variance (ANOVA) may be used if the data satisfy the assumptions of independence, normality of residuals, and homogeneity of variance. If a statistically significant difference is identified, an appropriate post hoc test may be conducted to determine which formulations differ. The significance level should be specified in the final manuscript, commonly at ($p < .05$). The statistical test and results should be reported according to the actual analysis performed; no statistical conclusions can be established from the formulation table alone.

RESEARCH RESULT

Evaluation of Hair Tonic Preparations

Organoleptic Test

The organoleptic test was conducted to examine the physical characteristics of the hair tonic preparations by direct observation, covering color, odor, and shape. The results for the three formulas are presented in Table 2. All formulas were white, had the characteristic odor of candlenut oil, and were liquid in form.

Table 2. Organoleptic Evaluation Results of Hair Tonic Formulas

Formula	Parameter	Result
F1 (candlenut oil 5 mL : VCO 5 mL)	Color	White
	Odor	Characteristic odor of candlenut oil
	Shape	Liquid
F2 (candlenut oil 5 mL : VCO 10 mL)	Color	White
	Odor	Characteristic odor of candlenut oil
	Shape	Liquid
F3 (candlenut oil 10 mL : VCO 5 mL)	Color	White
	Odor	Characteristic odor of candlenut oil
	Shape	Liquid

Source: Primary data, 2025

Hedonic Test

The hedonic test aimed to determine the panelists' level of preference for the color, aroma, and texture of each formula. The results are presented in Table 3. All three formulas (F1–F3) were liked in terms of color. For aroma, F1 and F2 were liked,

while F3 was rated slightly less favorably (somewhat disliked). F1 received the highest texture score among the test formulas (very liked).

Table 3. Hedonic Test Results of Hair Tonic Formulas

Criterion	Formula	Score	Conclusion
Color	Negative control (base)	1	Dislike
	F1	3	Like
	F2	3	Like
	F3	3	Like
	Positive control	4	Very like
Aroma	Negative control (base)	2	Somewhat dislike
	F1	3	Like
	F2	3	Like
	F3	2	Somewhat dislike
	Positive control	3	Like
Texture	Negative control (base)	3	Like
	F1	4	Very like
	F2	3	Like
	F3	3	Like
	Positive control	4	Very like

Source: Primary data, 2025

Homogeneity Test

The homogeneity test aimed to determine whether the ingredients of the hair tonic were uniformly mixed. All preparations (F1–F3) were homogeneous: the color was evenly distributed, with no particles settling and no phase separation. The results are presented in Table 4.

Table 4. Homogeneity Test Results of Hair Tonic Formulas

Formula	Parameter	Result
F1 (candlenut oil : VCO, 5:5)	Color evenly distributed throughout the preparation	Homogeneous
F2 (candlenut oil : VCO, 5:10)	Color evenly distributed throughout the preparation	Homogeneous
F3 (candlenut oil : VCO, 10:5)	Color evenly distributed throughout the preparation	Homogeneous

Source: Primary data, 2025

pH Test

The pH test aimed to determine the acidity level of the hair tonic formulas combining candlenut oil and virgin coconut oil (VCO). The pH of every preparation was 5 (Table 5). This is appropriate for topical scalp use, since a highly acidic pH can trigger skin irritation, whereas a highly alkaline pH can cause the skin to become dry and scaly. According to SNI 16-4954-1998, the recommended scalp pH ranges from 4.5 to 6.5; therefore, all formulas met this requirement.

Table 5. pH Test Results of Hair Tonic Formulas

Formula	Parameter	Result
F1 (candlenut oil : VCO, 5:5)	Preparation pH 4.5–6.5	5
F2 (candlenut oil : VCO, 5:10)	Preparation pH 4.5–6.5	5
F3 (candlenut oil : VCO, 10:5)	Preparation pH 4.5–6.5	5

Source: Primary data, 2025

Viscosity Test

The viscosity test aimed to determine the thickness of the hair tonic preparations, which influences their spreadability and ease of application on the scalp. All formulas (F1, F2, and F3) had a viscosity of 12 cPs, which is higher than the reference value of 5 cPs [18] (Table 6). The higher viscosity is likely due to the oil-based composition of the preparations. Because all three formulas gave the same value, the candlenut oil to VCO ratio did not visibly alter viscosity at the volumes tested.

Table 6. Viscosity Test Results of Hair Tonic Formulas

Formula	Result	Reference viscosity
F1 (candlenut oil : VCO, 5:5)	12 cPs	5 cPs [18]
F2 (candlenut oil : VCO, 5:10)	12 cPs	5 cPs [18]
F3 (candlenut oil : VCO, 10:5)	12 cPs	5 cPs [18]

Source: Primary data, 2025

Hair Growth Test in White Rats (*Rattus norvegicus*)

All treatment groups showed hair growth during the 28 days of treatment (Figure 1). The negative control (base without candlenut oil and VCO) had the lowest value (mean 2.77), followed by F3 (2:1; 4.22), F2 (1:2; 5.08), F1 (1:1; 7.64), and the positive control (commercial tea tree oil product; 8.89). The low value of the negative control shows that the base contributes little to hair growth, so the growth in the test groups can be attributed to the candlenut oil and VCO combination rather than to the base. Among the test formulas, F1 (1:1) had the highest mean value and was the closest to the positive control.

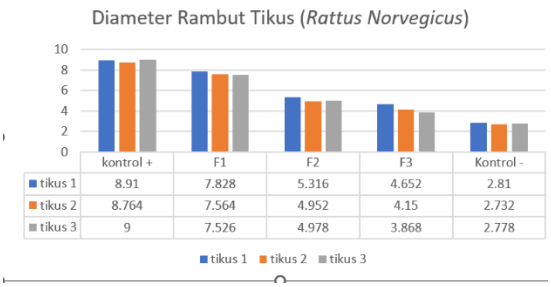


Figure 1. Hair growth of white rats (*Rattus norvegicus*) in each treatment group *(insert the bar chart: Rat 1–3 for K+, F1, F2, F3, and K−)*

Source: Primary data, 2025

Statistical analysis showed no significant effect of the candlenut oil to VCO ratio on hair growth in white rats, as indicated by a significance value above 0.05 (0.406 > 0.05) (Table 7). F1 (1:1; 5 mL candlenut oil and 5 mL VCO) is therefore considered the most optimum formula. It gave the highest mean growth among the test formulas, and its result did not differ significantly from F2 (1:2) and F3 (2:1), so the balanced 1:1 ratio is sufficient.

Table 7. Statistical Analysis of Differences in Hair Growth Among Formulas

Formula	Mean hair growth (mm)	Sig.	Remarks
Negative control (K−)	13	0.406	0.406 > 0.05: no significant difference among formulas
F1 (candlenut oil : VCO, 1:1)	14.2		
F2 (candlenut oil : VCO, 1:2)	14.2		
F3 (candlenut oil : VCO, 2:1)	11.7		
Positive control (K+)	14.2		

Source: Primary data, 2025

DISCUSSION

The findings indicate that all treatment groups exhibited hair growth during the 28-day observation period, although the reported mean values varied across the formulations. Formula I, containing 5 mL of candlenut oil and 5 mL of virgin coconut oil (VCO), produced the highest reported mean among the experimental formulations at 7.64, followed by Formula II, containing 5 mL of candlenut oil and 10 mL of VCO, at 5.08, and Formula III, containing 10 mL of candlenut oil and 5 mL of VCO, at 4.22. The negative control had a reported mean of 2.77, while the positive control achieved

the highest value of 8.89. Descriptively, these results suggest that Formula I may warrant further investigation as a potential hair tonic formulation. However, the statistical analysis yielded a significance value of 0.406, exceeding the 0.05 threshold. Therefore, the observed differences were not statistically significant, and the results do not establish that the three oil combinations differ in their effects on hair growth. Formula I's higher reported mean should consequently be interpreted as a descriptive observation rather than evidence of superior efficacy.

The potential contribution of VCO to the performance of the hair tonic may be considered in relation to its physicochemical characteristics and biological properties. Previous research has investigated VCO's composition, antioxidant activity, and potential protective effects in cellular and experimental models [8][9][10][11][12]. Illam et al. [8] reported that polyphenols in VCO could protect against pro-oxidant-mediated cell death, while Rohman et al. [9] reviewed its extraction methods, physicochemical properties, biological activities, and authentication. Ghani et al. [10] also demonstrated that processing methods are relevant to the physicochemical properties and antioxidant capacities of VCO. These findings provide a scientific basis for investigating VCO in topical formulations, but they do not directly demonstrate that VCO stimulates hair growth. Antioxidant or skin-protective activity should not automatically be interpreted as an effect on hair follicle cycling. Furthermore, the present study did not examine the biological mechanisms underlying the observed hair growth, nor did it evaluate the individual effects of candlenut oil and VCO. Therefore, the respective contributions of the two ingredients remain uncertain.

Formula I, which contained equal volumes of candlenut oil and VCO, produced the highest reported mean among the experimental formulations. One possible explanation is that the balanced combination may provide a different composition of lipid ingredients from formulations containing a higher proportion of either oil. Nevertheless, this explanation remains hypothetical because the chemical composition of the finished products, skin penetration, and hair follicle responses were not investigated. An important consideration is that the total oil volume differed between the formulations. Formula I contained 10 mL of combined oils, whereas Formulas II and III each contained 15 mL. Thus, the comparison involved differences in both the oil ratio and total oil content, making it difficult to attribute the observed variation exclusively to the relative proportions of candlenut oil and VCO. Research on VCO-based emulsions and lipid delivery systems has highlighted the importance of formulation design and the properties of the resulting preparation [2][6][13][14]. Future experiments should maintain a constant total oil concentration while systematically varying the ratio of the two ingredients. Additional treatment groups containing candlenut oil or VCO individually would help determine their separate effects and whether combining them produces an additional benefit. Accordingly, Formula I may be considered the most promising formulation based on the reported descriptive results, but its superiority and optimality have not been statistically established.

The organoleptic evaluation showed that all three formulations had a white appearance, a liquid consistency, and an odor characteristic of candlenut oil. These

findings indicate that the variations in oil proportions did not produce obvious differences in the recorded organoleptic characteristics. The hedonic evaluation provided additional information about panelists' preferences. All formulations were liked in terms of color, while Formulas I and II received favorable aroma ratings. Formula III was rated less favorably for aroma, potentially reflecting its higher proportion of candlenut oil; however, this explanation cannot be confirmed without further sensory or chemical analysis. Formula I also received the highest texture rating among the experimental formulations. These results suggest that Formula I had a comparatively favorable sensory profile, which may be relevant to the acceptability of a topical hair tonic. Nevertheless, sensory preference does not demonstrate hair growth efficacy or establish product safety. The number and characteristics of the panelists, the sensory scoring procedure, and the statistical analysis of the hedonic data should be reported to support a more robust comparison of acceptability.

The homogeneity test showed that all three formulations had evenly distributed color, with no visible sedimentation or phase separation. This indicates that the preparations appeared physically uniform at the time of evaluation. The pH of each formulation was 5, which falls within the scalp pH range of 4.5–6.5 cited in the study. A pH within this range may be appropriate for a topical scalp product, but pH alone cannot establish that a preparation is non-irritating or safe for repeated application. Additional assessments, including skin irritation and stability testing, are required before the formulations can be considered suitable for broader use. The viscosity test recorded a value of 12 cP for all three formulations, compared with the reference value of 5 cP cited from previous hair tonic research [18]. The higher measured viscosity may influence the spreading, dispensing, and distribution of the preparation on the scalp. However, whether 12 cP is unsuitable depends on the intended product characteristics and the validity and comparability of the reference method. Because the same viscosity was recorded for all three formulations, the results indicate that no difference was detected by the reported measurement. They do not establish that variations in oil proportions can never affect viscosity. Further evaluation should examine spreadability, physical stability, and changes in formulation characteristics during storage.

The control groups provide additional context for interpreting the hair growth results. The negative control had the lowest reported mean, whereas the positive control produced the highest. This descriptive pattern suggests that the observed outcomes varied between the base formulation, the experimental oil combinations, and the positive-control product. However, the difference cannot be attributed solely to candlenut oil and VCO because the base contained other ingredients, including ethanol, propylene glycol, PEG 400, Tween 80, and menthol. These components may influence the physical properties and delivery of the formulation. Furthermore, the positive control was identified as a commercial tea tree oil product, but its complete composition and comparability with the experimental formulations were not provided. Consequently, the positive control cannot be used to establish the specific contribution of candlenut oil or VCO. The statistical result of 0.406 also requires clarification regarding which groups were included in the analysis. If all

five groups were analyzed together, the result indicates that an overall statistically significant difference was not detected. If only the three experimental formulations were compared, the result cannot establish whether those formulations differed from either control. The final manuscript should specify the statistical test, groups included, assumptions assessed, and exact outcome of the analysis.

The findings should also be interpreted in light of the broader evidence concerning VCO. Previous experimental studies have examined its biological effects in animal models, including immune responses, bone oxidative status, and memory-related outcomes [3][15][16][17]. These studies demonstrate that VCO has been investigated in different physiological contexts, but their findings do not establish its effectiveness in promoting hair growth. Similarly, research on VCO's anti-inflammatory and skin-protective properties provides relevant background for topical product development without directly confirming a hair growth effect [11][12]. The present study therefore contributes preliminary observations on formulations combining candlenut oil and VCO, while the biological mechanisms and efficacy of these combinations remain unresolved. Direct assessment of hair growth, supported by standardized measurements and suitable controls, is necessary to determine whether the observed outcomes represent reproducible treatment effects.

Several methodological and reporting limitations need to be addressed before definitive conclusions can be drawn. First, the reported hair growth values in the narrative are inconsistent with those presented in Table 7. The narrative reports values of 7.64 for Formula I, 5.08 for Formula II, 4.22 for Formula III, 2.77 for the negative control, and 8.89 for the positive control, whereas Table 7 reports values of 14.2 for Formula I, Formula II, and the positive control, 11.7 for Formula III, and 13 for the negative control. These discrepancies must be resolved using the original dataset. The units and operational definition of the hair growth measurement should also be specified consistently. Second, information concerning the number of animals per group, animal characteristics, allocation procedures, treatment frequency, measurement intervals, and individual observations is insufficiently detailed. These factors are necessary to evaluate reproducibility and the reliability of the statistical analysis. Third, the methods used to assess statistical assumptions and determine the significance value should be reported clearly. Future studies should use an adequately justified sample size, standardized application and measurement procedures, a constant total oil concentration, and clearly defined control groups. Safety assessments and longer-term stability testing would also strengthen the evidence concerning the practical suitability of the formulations.

Overall, the study provides preliminary experimental observations on hair tonic formulations containing different combinations of candlenut oil and VCO in white rats (Rattus norvegicus). Formula I produced the highest reported mean among the experimental formulations and received favorable sensory ratings. Nevertheless, the statistical result of \(p = 0.406\) indicates that the observed differences were not statistically significant, so the superiority of Formula I has not been established. The formulations also showed similar recorded physical characteristics, including homogeneity, a pH of 5, and a viscosity of 12 cP. Further research should reconcile the inconsistent hair growth data, standardize

the total oil concentration, evaluate the individual effects of both oils, and use appropriate statistical procedures and adequately justified sample sizes. These steps are necessary to determine whether the combinations have a reproducible effect on hair growth and to establish their safety and suitability for future hair tonic development.

CONCLUSION

This study evaluated the comparative effects of candlenut oil and virgin coconut oil (VCO) combinations in hair tonic formulations on hair growth in white rats (Rattus norvegicus). Among the three experimental formulations, Formula I, containing 5 mL of candlenut oil and 5 mL of VCO, demonstrated the highest reported mean hair growth and favorable sensory characteristics. All formulations exhibited homogeneous preparations, a pH of 5, and a viscosity of 12 cP. However, the statistical analysis revealed no significant difference among the evaluated groups ($p = 0.406$), indicating that the superiority of Formula I could not be established statistically. Therefore, the findings provide preliminary evidence regarding the potential of these oil combinations but do not confirm their effectiveness in promoting hair growth. Future research should verify the consistency of the original data, standardize the total oil concentration, include adequate sample sizes and appropriate controls, and conduct further efficacy, safety, and stability assessments to establish the most suitable formulation.

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