

Irritation, hedonic, and hair growth effectiveness tests of black cumin (*Nigella sativa* L.) oil-based shampoo nanoemulsion

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Abstract

Background: Hair loss is a common dermatological problem in Indonesia that affects the quality of life of those affected. Black cumin oil (*Nigella sativa* L.) contains thymoquinone, essential fatty acids, and antioxidants that have the potential to inhibit hair loss while simultaneously stimulating hair growth. A nanoemulsion formulation was selected to enhance the penetration of active ingredients into the scalp more optimally.

Method: This experimental study involved 20 volunteer panelists, with assessments including a skin irritation test, hedonic test (aroma, texture, freshness, and comfort), and hair growth effectiveness test using a digital microscope on days 1, 3, 5, 7, 14, and 21.

Results: The irritation test yielded an average score of 0.1, with more than 95% of panelists showing no signs of irritation. The hedonic test demonstrated positive responses from all panelists across all evaluated parameters. Hair growth was first detected on day 5 and continued to develop evenly through day 21.

Conclusion: The black cumin oil nanoemulsion shampoo was proven to be safe, well-accepted organoleptically, and effective in stimulating hair growth, making it a promising natural-based hair care alternative.

Keywords: Hair Growth; Hedonic Test; Irritation; Nanoemulsion; *Nigella sativa*; Shampoo

INTRODUCTION

Hair Besides being a natural barrier and temperature regulator, hair plays a crucial role in enhancing a person's appearance and identity. Hair production is centered within the follicle, a microscopic sac located in the dermis of the skin. Anatomically, hair strands are filaments that protect the scalp from physical impact, ultraviolet radiation, and the adverse effects of weather. Beyond these biological functions, hair holds crucial cultural and aesthetic value for human self-image(1).

Therefore, hair thinning or loss often occurs due to damage to the strand structure, lack of nutrition, psychological stress, and free radicals in the scalp area. Based on data from the 2023 Basic Health Research (Riskesdas), the phenomenon of mental stress in adolescents aged 15 and above reached 6% (equivalent to 14 million individuals). In line with this condition, a public study found that hair loss is the highest problem in Indonesia, with a percentage reaching 64.7%, with this

phenomenon predominantly affecting the younger generation aged 20 to 25.(1)

As a cleaning product, shampoo is required to be optimally effective in various water conditions, be safe for the scalp, and not cause eye irritation. The ideal formula should produce abundant lather, be easy to rinse off, and leave no residue(2).The surfactants in it bind and lift away sebum and dirt buildup on hair. Economically, this cleansing product is generally marketed at a much more affordable price compared to hair tonic or conditioner.(3).

The modern cosmetics sector widely utilizes *Nigella sativa* L. seeds as a raw material for hair care because of its efficacy in reducing skin inflammation(4), and inhibit hair loss, and block the harmful effects of UV rays. The seeds contain a variety of biologically active components, including essential oils, thymoquinone, thymol dithymoquinone, thymohydroquinone, linoleic acid, oleic acid, lipids, sterols, and alkaloids(5).

The compound thymoquinone is known to protect hair follicles from oxidative damage and attacks by microbes that trigger baldness, while also strengthening the hair root and shaft tissue. This is supported by findings that black cumin oil extract, rich in oleic and linoleic acids, is effective in suppressing hair loss. This effectiveness can be maximized through innovation in more advanced drug carrier systems, one of which is through the engineering of nanoemulsions.(6).

Nanoemulsion technology utilizes a highly potent fat-based system to accelerate the penetration of active ingredients into skin pores. This mechanism enhances the solubility of hydrophobic compounds, facilitating absorption, and increasing bioavailability. Thanks to its extremely small particle size, the contact surface area and free energy are increased, resulting in a more even distribution of herbal nutrients and rapid absorption, optimally stimulating hair growth.(7)

Based on these advantages, developing research into herbal nanoemulsion shampoo products is crucial. Therefore, this literature review was initiated to identify the irritation and hedonic properties of the black cumin oil nanoemulsion shampoo formula, as well as its hair growth effectiveness.

METHOD

This research is an experimental laboratory study that aims to evaluate the preparation of black cumin (*Nigella Sativa* L.) oil shampoo nanoemulsion by conducting skin irritation tests and sensory hedonic tests or panelists' acceptance of the aroma, texture, freshness and comfort of the shampoo nanoemulsion preparation as well as the effectiveness of hair growth on each panelist.

The tools used in this study were irritation scale sheets (red, itchy allergies) and hedonic test scale sheets (aroma, texture, freshness and comfort), digital microscopes and laptops for documentation of the effectiveness of black cumin oil (*Nigella Sativa* L.) nanoemulsion shampoo preparations. The ingredients used were the F1 black cumin oil nanoemulsion formula

which was selected based on the best physical properties and characteristics of nanoparticles and formulated into a black cumin oil nanoemulsion shampoo with good physical properties of stability, high foam and foam stability during 1 month of storage at room temperature.

This research method begins with a test of the irritation of the nanoemulsion shampoo on human skin by applying the preparation to the surface of the skin behind the ear. The back of the ear is the same as the surface of the skin on the hair. With descriptive observation parameters of what reactions occur after using the nanoemulsion shampoo of black cumin oil (*Nigella Sativa* L.) then left for 15 minutes and recorded what changes occur in the panelist's skin. redness, itching and allergies. no irritation (0.0), very little irritation (0.1-0.4), little irritation (0.41-1.9), moderate irritation (2.0-4.9), severe irritation (5.0-8.0). This test was conducted on 20 panelists with the inclusion criteria of being willing to fill out a statement of willingness, volunteers aged 18-30 years old, men and women(8).

Hedonic testing of cumin oil-based nanoemulsion shampoo plays a crucial role in the cosmetic development process. Through this testing, we can determine the extent to which consumers like and accept various product characteristics, including the aroma, texture, and comfort when applied to the skin. Ultimately, this hedonic testing aspect determines whether a cosmetic product will survive and be accepted in the market.

Hedonic test was then conducted involving 20 panelists who were selected to provide subjective assessments of various sensory aspects of the nanoemulsion shampoo preparation containing black cumin (*Nigella sativa* L.) active ingredient. The sensory aspects assessed in this hedonic test included aroma, texture, freshness, and comfort of use of the shampoo preparation (8).

The assessment data collection process was carried out using two approaches, namely by asking each panelist directly and through a direct face-to-face interview session to obtain a more in-depth and representative response.(9). In addition, each panelist was also given a self-assessment sheet or Self Assessment Form which had to be filled out individually based on their personal experience and perception of the preparation being tested(10).

The assessment on the sheet uses an ordinal scale scoring system with a value range of 1 to 5, where a score of 5 indicates the category of really like, a score of 4 indicates the category of like, a score of 3 indicates the category of disagree, and a score of 2 indicates the category of disagree. A score of 3 indicates a neutral category, a score of 2 indicates a dislike category, and a score of 1 indicates a very dislike category. This scoring system is used to quantify the panelists' level of preference in a structured manner so that the data obtained can be analyzed statistically to evaluate the level of consumer acceptance and preference for the formulated black cumin oil shampoo nanoemulsion preparation(11).

Hair growth effectiveness was measured using a digital microscope as an observation tool. In this testing phase, panelists applied a shampoo preparation to areas of the scalp indicated as alopecia or hair loss, then performed a microscopic examination by gently placing the lens of the measuring instrument on predetermined test points on the scalp's surface. Resolution and visual acuity were adjusted by rotating or shifting the lens until the morphology of the follicle and hair shaft was precisely identified. The zoom scale was also adjusted to provide an in-depth projection of hair anatomy, including the root, hair shaft, and the initiation of new hair growth in the form of vellus hair (12).

When indications of hair growth are detected, the time or first day of its appearance will be recorded systematically referring to the established clinical scoring scale, namely a score of 0 for conditions that do not show any signs of hair growth, a score of 1 if the fine hair (vellus) hair phase begins to appear, a score of 2 for hair that is experiencing growth at a moderate level, a score of 3 if the distribution of hair growth is already evenly distributed, and a score of 4 for hair growth distribution, which is categorized as very even. Implementing this scoring method simplifies the process of reviewing and documenting test data for optimal results. This periodic monitoring is applied longitudinally on days 1, 3, 5, 7, 14, and 21 using the digital microscope(13).

RESULTS

Table 1. Results of Irritation Test on Panelists

No	Panelists Initials	Redness	Itching	Allergy
1.	SN	0,0	0,0	0,0
2.	AD	0,0	0,0	0,0
3.	INR	0,0	0,0	0,0
4.	SR	0,0	0,0	0,0
5.	FY	0,0	0,0	0,0
6.	JA	0,0	0,0	0,0
7.	BS	0,0	0,0	0,0
8.	RA	0,0	0,0	0,0
9.	ZA	0,0	0,0	0,0
10.	MR	0,0	0,0	0,0
11.	AA	0,0	0,0	0,0
12.	FI	0,0	0,0	0,0
13.	EL	0,0	0,0	0,0
14.	AR	0,0	0,0	0,0
15.	TL	0,0	0,0	0,0
16.	HA	0,0	0,0	0,0
17.	AP	0,1-0,4	0,0	0,0
18.	DWM	0,0	0,0	0,0
19.	NF	0,0	0,0	0,0
20.	BH	0,0	0,0	0,0
Total Score		0,1	0,0	0,0

Description: no irritation (0.0), very slight irritation (0.1-0.4), slight irritation (0.41-1.9), moderate irritation (2.0-4.9), severe irritation (5.0-8.0)

Table 2. Results of Panelist Hedonic Tests on Aroma, Texture, Freshness and Comfort

Panelists Initials	Aroma					Texture					Freshness					Comfort				
	SS	S	N	TS	STS	SS	S	N	TS	STS	SS	S	N	TS	STS	SS	S	N	TS	STS
SN	√	-	-	-	-	-	-	√	-	-	√	-	-	-	-	√	-	-	-	-
AD	-	√	-	-	-	√	-	-	-	-	√	-	-	-	-	-	√	-	-	-
INR	√	-	-	-	-	-	√	-	-	-	-	√	-	-	-	√	-	-	-	-
SR	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-	-	√	-	-	-
FY	-	√	-	-	-	√	-	-	-	-	-	-	√	-	-	√	-	-	-	-
JA	√	-	-	-	-	√	-	-	-	-	√	-	-	-	-	√	-	-	-	-
BS	-	√	-	-	-	-	√	-	-	-	-	√	-	-	-	√	-	-	-	-
RA	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-	-	√	-	-	-
ZA	√	-	-	-	-	√	-	-	-	-	√	-	-	-	-	√	-	-	-	-
MR	√	-	-	-	-	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-
AA	-	√	-	-	-	-	√	-	-	-	√	-	-	-	-	-	√	-	-	-
FI	√	-	-	-	-	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-
EL	-	√	-	-	-	√	-	-	-	-	√	-	-	-	-	-	√	-	-	-
AR	-	√	-	-	-	√	-	-	-	-	√	-	-	-	-	√	-	-	-	-
TL	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-	-	√	-	-	-
HA	-	√	-	-	-	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-
AP	√	-	-	-	-	-	√	-	-	-	-	√	-	-	-	-	√	-	-	-
DWM	-	√	-	-	-	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-
NF	√	-	-	-	-	-	√	-	-	-	√	-	-	-	-	-	√	-	-	-
BH	√	-	-	-	-	√	-	-	-	-	√	-	-	-	-	√	-	-	-	-
Total	12	8	0	0	0	11	8	1	0	0	12	7	1	0	0	12	8	0	0	0
%	60	40	0	0	0	55	40	5	0	0	60	35	5	0	0	60	40	0	0	0

Description: Score 5 = Like Very Much (SS), Score 4 = Like (S), Score 3 = Neutral (N), Score 2 = Dislike (TS), Score 1 = Dislike Very Much (STS)

Table 3. Results of Hair Growth Effectiveness Tests on Panelists

Panelists Initials	Day 1	Day 3	Day 5	Day 7	Day 14	Day 21
SN	0	0	1	1	2	3
AD	0	0	1	2	2	3
INR	0	0	1	2	3	3
SR	0	0	1	1	2	2
FY	0	0	1	2	3	4
JA	0	0	0	1	2	3
BS	0	0	1	1	2	2
RA	0	0	1	2	3	4
ZA	0	0	0	1	2	3
MR	0	0	1	2	2	2
AA	0	0	1	1	2	4
FI	0	0	0	1	2	3
EL	0	0	1	2	3	3
AR	0	0	1	1	2	3
TL	0	0	0	1	2	2
HA	0	0	0	2	3	4
AP	0	0	1	1	2	3
DWM	0	0	1	1	2	2
NF	0	0	0	2	3	4
BH	0	0	1	1	2	3

Description: no growth = (0), fine growth = (1), moderate growth = (2), even growth = (3), very even growth = (4)



Figure 1. Irritation Test Results



Figure 2. Hedonic Test Results

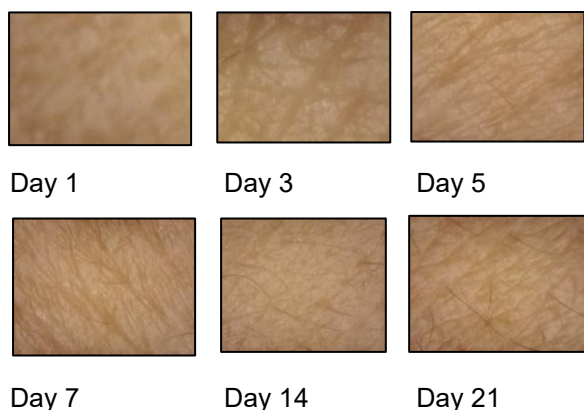
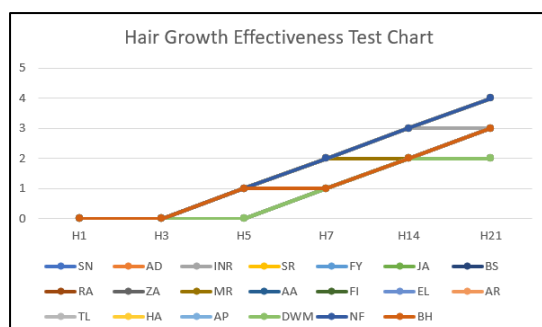


Figure 3. Hair Growth Microscope Test Results from Panelists



Description: No Growth = (0), Fine Growth = (1), Moderate Growth = (2), Even Growth = (3), Very Even Growth = (4)

Figure 4. Hair Growth Effectiveness Test Results Graph

DISCUSSION

Irritation testing is carried out to determine the safety of the preparation and to find out whether the preparation can cause negative reactions to the skin or tissue such as the parameters in the test results table based on the code of ethics permit letter with (Number: 4788 / UN21.8 / PT.01.04 / 2025) on 20 panelists so that the score results in table 1 are 0.1. The value obtained is included in the category that does not cause irritation because from the irritation test results table it can be seen that many or most of the panelists showed a score of 0.0. This indicates that after using black cumin (*Nigella Sativa* L.) oil shampoo nanoemulsion, it does not cause irritation such as redness, itching, and allergies. Based on previous research by Hariyani et al., (2026). Reporting that a score of 0.1-1.9 is still considered mild irritation. Of all the subjects observed, only one panelist reported minor irritation symptoms in the form of very mild redness. The evaluation results showed a score index between 0.1 and 0.4, which means it still falls into the very mild irritation classification. Given that these side effects are very minimal and not harmful, the panelist's skin is confirmed to be safe and does not require further medical intervention or treatment(11).

These results indicate that the black cumin oil shampoo nanoemulsion formulation exhibits an optimal safety profile and good scalp tolerability. Based on research by Lestari et al., (2025). This aligns with the irritation test parameters, which define the category "safe for application to the scalp" if the percentage of respondents free from irritation symptoms reaches more than 80%. The safety characteristics of this preparation are influenced by the use of black cumin essential oil at a concentration maintained at 0.5%, which is still within safe limits. Furthermore, the integration of excipients such as glycerin and purified water, which act as solvents and guarantee moisture, also contributes to minimizing the potential for irritation to skin tissue(6).

The nanoscale particle size facilitates a more homogeneous and stable distribution of active ingredients over the skin's surface, while minimizing the risk of toxicity. These

characteristics align with previous literature findings showing that nanoparticle-based formulations can enhance the effectiveness of bioactive compounds. Furthermore, this system has been shown to reduce potential irritation thanks to its ingredient release mechanism, active which takes place in a more controlled manner (14).

The black cumin (*Nigella Sativa* L.) oil shampoo nanoemulsion preparation is safe to use because it does not cause high category irritation in the parameters observed and felt by the panelists.

A hedonic test was conducted to determine the extent to which panelists accepted and preferred a black cumin (*Nigella sativa* L.) oil shampoo nanoemulsion. This test was intended to assess various sensory aspects of the product that directly influence panelists' comfort and preference in its use. The results of the hedonic test from 20 panelists are shown in Table 2(8).

To measure the extent to which the panelists liked and accepted the black cumin oil (*Nigella Sativa* L.) nanoemulsion shampoo preparation, a hedonic test was conducted which included parameters of aroma, texture and freshness effect as well as comfort of use (8). The data in table II shows that this nanoemulsion shampoo formula received a very good response from all panelists in all aspects of the assessment, interestingly none of the panelists chose the option stating that they did not like or really did not like which proves that this preparation was well accepted by the panelists.

Through the hedonic test, it can be determined to what extent the nanoemulsion formulation of black cumin oil (*Nigella sativa* L.) is proven to be effective in meeting panelists' preferences and can serve as a basis for improving the sensory aesthetic aspects of the product. Therefore, this test is not only for quality evaluation, but also categorized as an initial step in the development of products or cosmetic preparations made from natural ingredients that can attract public interest(15).

The percentage of aroma shows the results of 12 panelists and a percentage of 60% really like it, while 8 panelists with a

percentage of 40% stated that they like the aroma of this black cumin oil nanoemulsion. The aroma preferred by these panelists shows the volatile characteristics of black cumin essential oil, which can provide a fresh fragrance without giving a strong odor and an unpleasant aroma. Aroma is one of the important sensory aspects in shampoo preparations because it provides the first impression that is immediately felt by the user when the product is used. A pleasant aroma can increase user comfort and satisfaction during the shampooing process, thereby influencing consumer acceptance and preference for the product. In addition, aroma also plays a role in building a positive perception of the quality and effectiveness of the shampoo. In the context of preparations containing the active ingredient black cumin oil (*Nigella sativa* L.), the right concentration will provide a fresh aroma(16).

From the texture of the black cumin oil shampoo nanoemulsion, the results showed that 11 people with a percentage of 55% of panelists stated that they really liked the texture of the shampoo nanoemulsion because it was easy to pour and apply and 8 people as many as 40% of panelists liked it and 1 person with a value of 5% neutral value entered the acceptable category because they still liked the preparation. The smoothness of the texture when applied is due to the use of Tween 80 and PEG which are able to form a homogeneous nanoemulsion system and stable stability and are easy to spread evenly(17).

From the freshness aspect, the percentage of results shows that 12 people with 60% of panelists really like the freshness of this preparation due to the sensation of the flavoring agent peppermint oil and black cumin essential oil as well as from the formulation components of this black cumin oil nanoemulsion preparation and 7 people 35% like the freshness of the nanoemulsion preparation and 1 panelist with a percentage of 5% is neutral towards the freshness of the shampoo nanoemulsion preparation. The fresh sensation after shampooing provides a strong psychological signal to consumers that their hair and scalp have been optimally freed

from the accumulation of sebum and dirt. In addition to the sensory effects. Based on previous research by Ham et al., (2024), the addition of refreshing agents or volatile components of essential oils such as Peppermint and *Nigella sativa* actively stimulate cold receptors in the scalp to trigger mild local vasodilation, which physiologically improves blood circulation around hair follicles. This improved blood flow not only optimizes nutrient distribution to hair roots, but the resulting cooling and refreshing effect also acts as a natural antipruritic, effectively diverting itch signals from the scalp's nervous system, a symptom that often accompanies hair loss(18).

For the comfort aspect of use, 60% of 12 panelists stated that they really liked it, giving a comfortable feeling that felt cool on the skin, then 40% of 8 panelists stated that they liked the comfort when applied. Where the results of the panelists did not give a dislike or very dislike, this shows that the panelists felt comfortable using the preparation and without any sensations that harm the panelists such as itching, allergies and burning sensations when using it. This good comfort shows that the components and formulation and concentration of black cumin oil are appropriate and do not cause negative reactions in the panelists.(19).

The data presented in Table 3 illustrates the visualization and clinical significance of hair growth development in 20 panelists, who were periodically evaluated using a digital microscope over a 21-day retention period (days 1, 3, 5, 7, 14, and 21). Based on the quantitative data, the development graph shows a consistent positive trend in the majority of test participants. In the initial observation phase, namely days 1 and 3, the preparation's efficacy did not show significant morphological changes, with the hair follicle assessment scores of most panelists remaining in the low or stagnant category. This is a normal physiological condition, considering that follicles need time to absorb the active ingredient.(20).

However, entering the medium to late term observation phase (day 5 to day 21), stimulation of new hair growth began to be

detected and measured in 14 individuals. This progress was marked by the emergence of new hair shoots that were still fine and clearly observed through microscopic scanning. This biological phenomenon is strong empirical evidence that the black cumin (*Nigella sativa* L.) oil-based shampoo nanoemulsion preparation has effective percutaneous penetration capabilities, so it is able to work actively at the cellular level to trigger and accelerate the anagen phase (active growth) of hair(21).

CONCLUSIONS

Based on the research results, it can be concluded that the black cumin (*Nigella sativa* L.) oil-based nanoemulsion shampoo preparation is proven to be safe, very well accepted by consumers, and effective in stimulating hair growth. This formulation has an optimal safety profile without triggering irritation reactions on the scalp. In addition, the sensory characteristics of the shampoo, including aroma, texture, freshness, and comfort of use, successfully obtained a high level of satisfaction from the panelists. Periodic evaluations also confirmed the preparation's efficacy in stimulating hair growth gradually, which is characterized by the emergence of new hair shoots. Thus, the use of this nanoemulsion technology has proven successful in optimizing the potential of black cumin oil as a safe and representative hair growth stimulator agent.

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