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Original Article

FORMULATION DEVELOPMENT AND STABILITY OF *MORINGA OLEIFERA* SEED OIL NANOEMULSION-BASED HAIR SERUM

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ABSTRACT

Objective: Synthetic drugs for treating hair loss often cause side effects. *M. oleifera* seed oil has shown potential in preventing hair loss; however, its oily and sticky texture limits its practicality for application. This study aims to develop a well-formulated and stable nanoemulsion-based hair serum containing *M. oleifera* seed oil at 4 °C and 40 °C.

Methods: The study utilized hair serum formulations containing nanoemulsified *M. oleifera* seed oil at concentrations of 10%, 20%, and 30%. The physical characteristics, including organoleptic properties, pH, viscosity, spreadability, and adhesiveness, were evaluated. Additionally, stability testing was conducted using a cycling test at 4 °C and 40 °C over six cycles.

Results: Variations in nanoemulsion concentration in the hair serum influenced pH, viscosity, adhesiveness, and spreadability, all of which met the required criteria. Higher concentrations of nanoemulsified *M. oleifera* seed oil resulted in lower pH, viscosity, and adhesiveness while increasing spreadability. Stability testing showed no statistically significant differences ($p>0.05$) in pH, viscosity, adhesiveness, or spreadability before and after the test.

Conclusion: The nanoemulsion-based hair serum formulation remained stable and effectively reduced the sticky and oily texture of *M. oleifera* seed oil, making it a more practical alternative for hair loss treatment.

Keywords: Nanoemulsion-based hair serum, *M. oleifera* seed oil, Formulation stability, Hair loss treatment

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INTRODUCTION

Hair loss and androgenetic alopecia are among the factors that affect quality of life and self-confidence [1]. One of the characteristics of androgenetic alopecia is follicular miniaturization, which is primarily influenced by genetic factors [2]. Androgenetic alopecia affects up to 98.6% of men and 64.4% of women, presenting with a distinct pattern of hair loss [3]. Based on data more than 50% of American men and 21.3% of Chinese men are prone to andogenous alopecia [4]. The pathogenesis of androgenetic alopecia can also be attributed to autoimmune processes, environmental factors, diseases, medications, oxidative stress, and stress potential [5]. This condition negatively impacts the antioxidant balance in both the blood and skin tissues. Clinical treatments for alopecia typically focus on enhancing immune function through various therapeutic approaches, including phototherapy and topical steroids. Additionally, immunosuppressants such as methotrexate and cyclosporine, as well as the use of vitamins and essential nutrients, are expected to mitigate oxidative stress [6]. The human scalp contains over 100,000 hair follicles, which play a crucial role in producing long and thick hair [7]. Hair serves several vital functions, including protection against cold temperatures, defining appearance and gender differences, and contributing to self-defense. The presence of dihydrotestosterone (DHT) receptors in hair follicles can lead to the shrinkage of dermal papilla stem cells, thereby resulting in incomplete follicular development [5]. One approach to reducing hair loss involves activating the hair roots [8].

One of the cosmetic products that can be used to address hair loss is hair oil. *M. oleifera* seed oil, also known as *M. oleifera* seed oil, is commonly utilized in the perfume and cosmetics industry [9]. Based on the research of Korassa *et al.* (2023), *M. oleifera* seed oil with a concentration of 12.5% has optimal activity as antialopecia [10]. *M. oleifera* seed oil contains unsaturated fatty acids such as oleic acid, stigmasterol, and β-sitosterol [11]. The phytosterol compounds found in *M. oleifera* seed oil include brassicasterol, ergostadienol, 24-methylenecholesterol, campasterol, campestanol, stigmasterol, ergostadienol, cholesterol, β-sitosterol, stigmastanol, 7-avenasterol, stigmastadienol, 28-isoavenasterol, and stigmastenol [12]. Some phytosterols, such as β-sitosterol, stigmasterol, and campasterol, can inhibit DHT production [13]. Furthermore, phytosterol concentrations ranging from 0.01% to 0.5% have been proven effective in preventing baldness [14]. Other components of *M. oleifera* seed oil include lauric acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid, and arachidic acid. The fatty acids found in wet-extracted *M. oleifera* seed oil include palmitoleic acid, palmitic acid, oleic acid, stearic acid, eicosenoic acid, arachidic acid, behenic acid, and lignoceric acid [15]. Linoleic acid in *M. oleifera* seed oil can stimulate hair growth through the inhibition of β-catenin and dickopf-related protein (DKK), both of which contribute to hair loss [16]. Oleic acid (more than 70%) is the most dominant fatty acid in *M. oleifera* seed oil [17]. Oleic acid in *M. oleifera* seed oil promotes hair growth by accelerating anagen phase production in hair follicles through the activation of the Wnt/β-catenin signaling pathway [18].

Based on biological data, *M. oleifera* seed oil is a promising candidate for stimulating hair growth. However, direct application of the oil can result in a greasy and sticky sensation. Currently, hair care trends emphasize the use of natural and herbal products, as they are believed to enhance vitality and overall health while minimizing exposure to potentially harmful chemicals [7]. Topical formulations for hair treatment must exhibit good skin permeability and be able to accumulate within hair follicles [19]. Research by Korassa *et al.* (2022) showed that *M. oleifera* seed oil hair tonic preparations with a concentration of 7.5%; 10% and 12.5% have good stability and meet the requirements [20]. Encapsulation of active compounds into nanoparticles or microparticles is able to enhance targeted delivery to hair follicles [21]. One promising nanoparticle type for improving skin penetration is nanoemulsion. Nanoemulsions serve as carriers with a transparent appearance and greater stability compared to conventional emulsions. Additionally, nanoemulsions increase surface area, thereby facilitating better penetration of active ingredients [22]. Nanoemulsion-based delivery systems offer enhanced biocompatibility, biodegradability, and extended shelf life [23].

The formulation of a herbal-based hair growth serum is expected to integrate traditional knowledge with modern scientific insights, ultimately addressing hair-related issues such as thinning and impaired hair growth [24]. Hair serum formulations are characterized by a high concentration of active ingredients, allowing for deeper and more intensive nourishment of the skin layers. Additionally, such formulations are anticipated to produce a non-greasy final product, making them more practical and suitable for application [19]. To the best of our knowledge, there is no formulation of *M. oleifera* seed oil in the form of nanoemulsion-based hair serum used for hair growth. The purpose of this study is to develop a nanoemulsion-based hair serum formula from *M. oleifera* seed oil to be developed as a hair growth product.

MATERIALS AND METHODS

Materials and equipment

M. oleifera seed oil was sourced from Blora, Central Java. Other materials included Tween 80, polyethylene glycol (PEG) 400, propylene glycol, hydroxypropyl methylcellulose (HPMC), methylparaben, propylparaben, butylated hydroxytoluene (BHT), and distilled water (Bratachem Indonesia). The equipment used in this study included a Particle Size Analyzer (PSA) (HORIBA SZ-100), pH meter (Trans Instrument HP 9000), sonicator (Elma Transsonic 570), and Transmission Electron Microscope (TEM) (JEOL/EO JEM-1400 version 1.0).

Methods

Extraction of *M. oleifera* seed oil

M. oleifera seed oil obtained from Blora, Central Java, was extracted using the cold-pressing method. The harvested *M. oleifera* seeds were peeled to remove their outer shells. The resulting kernels were then processed using a cold press. The extracted oil was stored in a tightly sealed container [25].

Preparation of nanoemulsion and *M. oleifera* seed oil nanoemulsion-based hair serum

Three variations of *M. oleifera* seed oil nanoemulsion-based hair serum were formulated based on the compositions listed in table 1. The nanoemulsion-based hair serum consisted of an oil phase, surfactant, and co-surfactant, with the optimal formula determined using the Simplex Lattice Design method. The optimal formulation was mixed until homogeneous using a magnetic stirrer at 800 rpm for 10 min at 25 °C. The mixture was then homogenized using a sonicator homogenizer for 10 min in two cycles. Buthylated hydroxytoluene (BHT), methylparaben, and propylparaben were added to the mixture. Propylene glycol, used as a thickening agent, was dissolved in water. Subsequently, HPMC, pre-dispersed in hot water (20 times the amount of HPMC), was incorporated and homogenized. The aqueous phase was then gradually added in a controlled manner according to the specified amount. Finally, the formulation underwent physical characteristic evaluations [26].

Physical evaluation

The physical evaluation test was conducted by examining the texture, color, and aroma of the nanoemulsion-based hair serum. The physical appearance of the formulation was assessed.

Homogeneity test

The homogeneity test was performed by observing the presence of clumps, flocculation, or aggregates in the nanoemulsion-based hair serum.

pH measurement

pH determination using a digital pH meter [27]. The pH measurement of the formulated nanoemulsion-based hair serum was carried out using a calibrated pH meter. Calibration was performed with pH 4.01 and pH 7.00 buffer solutions to ensure accurate functionality. The clean pH meter electrode was immersed in a vial containing the nanoemulsion-based hair serum formulation, and the pH value was displayed on the screen. The measurement was conducted at room temperature [7].

Viscosity test

The viscosity measurement was performed using a Brookfield viscometer equipped with a spindle 62 and 100 rpm [28]. The nanoemulsion-based hair serum formulation was placed in a glass beaker, and its viscosity was measured [24]. Evaluation were conducted in five replication.

Spreadability test

The spreadability test was conducted using an instrument typically employed to assess and measure the spreadability of semisolid formulations. One g of the nanoemulsion-based hair serum was placed between two flat plates measuring 20×20 cm, with a specific weight applied on top. The spread diameter was measured after 1 minute under the applied load [5].

Adhesion test

The adhesion test was conducted by placing 1 g of the formulation on a glass slide with a known surface area. Another glass slide was placed on top of the sample, followed by applying a 1 kg weight for 5 min. Afterward, an 80 g weight was released, and the time required for the two glass slides to separate was recorded. An optimal adhesion time for topical formulations is no less than 4 sec [29].

Stability test

The stability of the *M. oleifera* seed oil nanoemulsion-based hair serum was analyzed by storing at the *Climatic Chamber* the samples under fluctuating temperature conditions. The samples were stored at 4 °C, followed by 40 °C for six cycles. Respectively with 24 h for each cycle [30]. The results of observations of serum nanoemulsion based hair serum preparations are conducted before and after the cycling test for six cycles. Nanoemulison based hair serum preparations were performed in a one cycle process, in which they are stored at a cold temperature of 4 °C for 24 h and then placed at a temperature of 40 °C. All these series of processes are counted as one cycle. After completing six cycles, the physical characteristics of the nanoemulsion serum were observed. Tests carried out include organoleptical tests, pH, viscosity, spreadability, adhesion. The evaluated parameters included the absence of phase separation, inversion, aggregation, creaming, and coalescence in the formulation [18].

Table 1: The composition of *M. oleifera* seed oil nanoemulsion-based hair serum

Matreial	Function	K1 (% w/v)	K2 (% w/v)	K3 (% w/v)	Control (% w/v)
Nanoemulsion	Active substance	10	20	30	0
HPMC	Gelling agent	0.6	0.6	0.6	0.6
Propylenglycol	Humectan	10	10	10	10
Methyl paraben	Preservative	0.2	0.2	0.2	0.2
Propyl paraben	Preservative	0.02	0.02	0.02	0.02
BHT	Antioxidant	0.02	0.02	0.02	0.02
Water Ad	Solvent	100	100	100	100

The hair serum nanoemulsion formula contains K1: 10%, K2: 20%, and K3: 30% nanoemulsions

Data analysis

The results are presented as mean±standard deviation (SD). The data were then analyzed using ANOVA with a significance level of 0.05.

RESULTS

Based on previous research, it shows that *M. oleifera* seed oil contains 12 types of fatty acids with the largest oleic acid content of 71.52%. The optimal ratio for *M. oleifera* seed oil, Tween 80 as a surfactant, and PEG 400 as a co-surfactant is 6.401%, 36%, and 18.599%, respectively. The use of tween 80 as a surfactant, the higher concentration of tween 80 can enhance clarity, transmittance value and smaller particle size [31]. Meanwhile, the use of PEG 400 can increase the stability of nanoemulsions because it can optimizing the emulsification process in nanoemulsion preparations [32]. The optimal nanoemulsion formulation exhibits a liquid, slightly viscous consistency, a transparent yellow color, and is odorless (fig. 1). The results indicate that the *M. oleifera* seed oil nanoemulsion has a particle size of 237.06 nm. Transmission electron microscopy (TEM) analysis revealed the morphological characteristics of the nanoemulsion, particle size was found to be in the range of 200 nm (fig. 1c), a polydispersity index (PDI) value of 0.467, a zeta potential of-20.89 mV, and a transmittance of 97.7%.

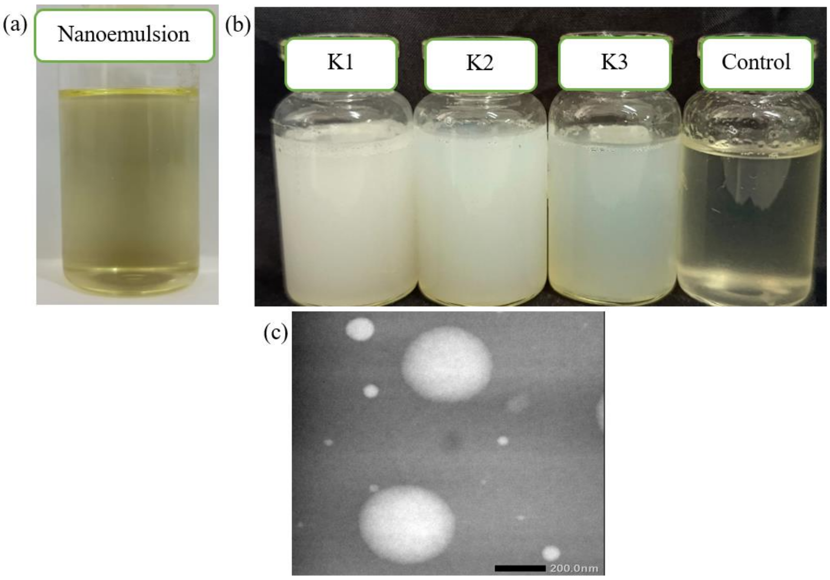

Fig. 1: Dosage forms a. nanoemulsion optimal formula; b. *M. oleifera* seed oil nanoemulsion-based hair serum; c. Particle size distribution and morphology of nanoemulsion use TEM 20.000 times (Scale bar 200 nm)

The nanoemulsion-based hair serum formulation underwent an organoleptic evaluation and exhibited organoleptic characteristics of a yellowish-white color and an odorless nature (fig. 1b). Visual inspection confirmed the absence of flocculation, clumping, or aggregation in the nanoemulsion based hair serum formulation. The addition of a certain amount of nanoemulsion to the serum base did not change the appearance of the nanoemulsion based hair serum. The homogeneity of the formulation was also well maintained. The pH evaluation is a crucial parameter in determining user comfort during application. The pH values are presented in table 2. All formulations (Formula 1 to 3) and the control (base formulation) exhibited slightly acidic pH levels, ranging from 5.59 to 6.96, which are close to the normal pH range.

Viscosity determination

The viscosity test results for the nanoemulsion based hair serum formulations (Formulas 1 to 3) ranged from 174.18 to 240.80 cPs. In comparison, the control formulation exhibited a higher viscosity of 276.40 cPs. The complete viscosity data are presented in table 2.

Spreadability and adhesiveness

The spreadability and adhesiveness test results are summarized in table 2. The spreadability of nanoemulsion based hair serum formulations 1 to 3 ranged from 9.54 to 10.34 cm, whereas the control formulation exhibited a spreadability of 8.90 cm. The adhesiveness of Formulas 1 to 3 ranged from 35.82 to 50.40 sec.

Table 2: Physical characteristics of *M. oleifera* seed oil nanoemulsion based hair serum

Formula	Organoleptic	pH	Viskosity (cPs)	Adhesive test (sec)	Spreadability
K1	liquid, yellowish white, homogeneous	6.57±0.13*	240.80±19.32*	50.40±2.07	9.54±0.15*
K2	liquid, yellowish white, homogeneous	6.27±0.07*	208.98±5.35*	42.40±3.05*	10.06±0.19*
K3	liquid, yellowish white, homogeneous	5.59±0.22*	174.18±20.68*	35.82±1.49*	10.34±0.15*
Control	liquid, yellowish white, homogeneous	6.96±0.07	276.40±13.74	53.60±1.67	8.90±0.19

Data presented as mean±standard deviation (SD) and n = 5. *p value>0.05

Formulation stability

The stability evaluation of the *M. oleifera* seed oil nanoemulsion based hair serum was conducted using a cycling test for six cycles at storage temperatures of 4 °C and 40 °C. The results indicated no significant changes in the physical properties, including color, odor, and consistency. The test results show that the pH of the preparation tends to increase (fig. 2a). During storage, the viscosity of the nanoemulsion based hair serum preparation decreased for formulas K2 and K3 as well as the control, but for formula K1, the viscosity increased (fig. 2b). The results of the stability test of the spreadability of the preparation are inversely proportional to the viscosity. Fig. 2c shows that the spreadability of formulas K1 and K3 has decreased, while formula K2 has increased. Based on fig. 2d, it shows that formulas K1 and K2 as well as the control, experienced a decrease in stickiness. However, formula K3 experienced an increase in adhesion.

DISCUSSION

Nanoemulsions are thermodynamically stable drug delivery systems with a transparent visual appearance. The particle size of nanoemulsions ranges from 20 to 500 nm and consists of an oil phase, a surfactant, and a co-surfactant. Nanoemulsions are commonly used to reduce the greasy feel of hair. Additionally, they are widely utilized due to their low viscosity, stability, and ability to enhance the solubility of active ingredients. Nanoemulsions are frequently incorporated into hair conditioners, shampoos, deodorants, sunscreens, and hair serums [33]. *M. oleifera* seed oil nanoemulsion exhibits a transparent appearance with no sedimentation. Its particle size of 237.06 nm aligns with nanoemulsion characteristics. Polydispersity index (PDI) was measured using the Malvern Zetasizer based on time-dependent light scattering, reflecting the principle that smaller particles diffuse more rapidly than larger ones [34]. A PDI value of 0.467 indicates a homogeneous particle size distribution. PDI values ranging from 0.01 to 0.5 reflect a narrow size distribution, while values above 0.7 indicate a very broad distribution [35]. The resulting PDI value of the nanoemulsion was below 0.5, indicating a homogeneous particle distribution. The zeta potential of the nanoemulsion was measured at-20.89 mV, indicating a negative surface charge, which can be attributed to the presence of negatively charged surfactants and cosurfactants [36]. The surfactant phase, in this case, Tween 80, reduces and stabilizes interfacial tension [37]. Moreover, surfactants facilitate spontaneous nanoemulsion dispersion and prevent coalescence. The co-surfactant used, PEG 400, reinforces surfactant molecules, further tightening interfacial tension. Additionally, co-surfactants enhance the interfacial fluidity of emulsions [38].

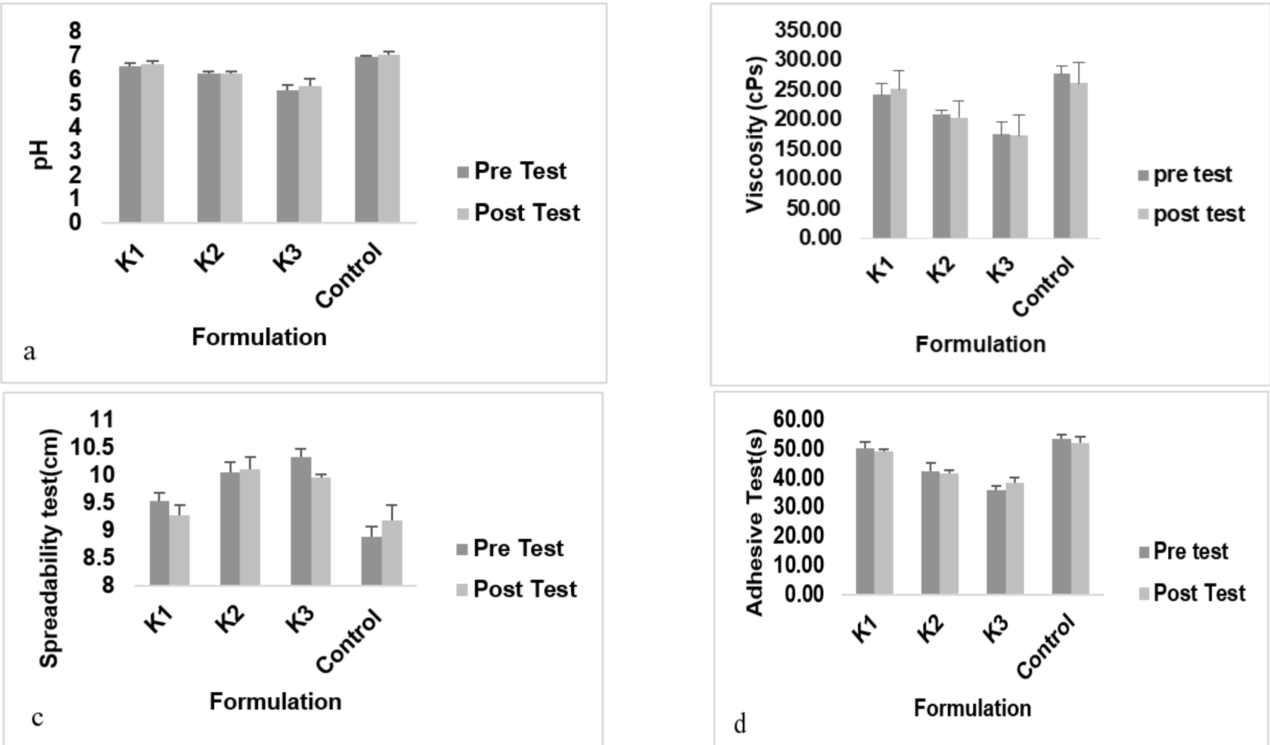

Fig. 2: Stability test results of nanoemulsion based hair serum before and after cycling test. a. pH test; b: viscosity test; c: spreadability test; d: adhesion test, data are presented as mean±standard deviation (SD). ns: not significant

The nanoemulsion based hair serum formulation was obtained by incorporating the nanoemulsion into a gel-based serum. The *M. oleifera* seed oil nanoemulsion based hair serum consists of several components, including a gelling agent, preservative, antioxidant, and humectant. Hydroxypropyl methylcellulose (HPMC) serves as the gelling agent, forming a hydrogel that can disperse in water [39]. This ingredient contributes to the viscosity, influencing the physical properties of the serum. In addition to the gelling agent, methylparaben and propylparaben are included as preservatives. These compounds help prevent bacterial contamination, which may occur due to the high water content in the nanoemulsion based hair serum [40]. Butylated hydroxytoluene (BHT) is used as an antioxidant, while propylene glycol functions as a humectant [40].

Based on the formulation and evaluation results, all nanoemulsion based hair serum formulations containing *M. oleifera* seed oil exhibited a slightly viscous and homogeneous texture. This evaluation was conducted to assess patient acceptance, which is crucial for enhancing product appeal and adherence key factors in the success of pharmaceutical innovation and formulation [41]. The pH measurement aimed to determine whether the formulation's pH aligns with that of the scalp. Topical formulations should have an optimal pH range of 4.5–6.5 to prevent irritation and ensure proper scalp maintenance [42]. The pH test results indicated that higher nanoemulsion concentrations corresponded to lower (more acidic) pH values. This pH reduction may be attributed to the auto-oxidation of Tween 80 [37]. According to Kishore *et al.* (2011), Tween 80 can undergo hydrolysis, producing fatty acids, which increase the concentration of dissociated H+ions, leading to a lower pH value [43]. However, all three formulations remained within the safe pH range for topical hair applications, as specified by the Indonesian National Standard (SNI 16-4399-1996). Formulations that are excessively acidic may cause scalp irritation, while those that are too alkaline can lead to dryness and flaking [44]. Statistical analysis using ANOVA revealed significant differences among all formulations ($p<0.05$), indicating that variations in concentration influenced the pH values. When compared to the control, all formulations exhibited statistically significant differences.

The viscosity determination aims to assess the texture and thickness of the nanoemulsion based hair serum formulation, which is related to its adhesiveness and spreadability when applied to the scalp. The viscosity range for Formulations 1 to 3 was 174.18–240.80 cPs, meeting the criteria for an optimal viscosity level. ANOVA results indicated a significant difference ($p \leq 0.05$) in viscosity, with all formulations showing statistically significant differences compared to the control. This suggests that increasing the nanoemulsion concentration alters viscosity. Viscosity plays a crucial role in formulation stability, application, and drug release. An optimal viscosity level ensures smooth distribution, enhances formulation performance, and prevents phase separation, ultimately improving the therapeutic application of a cosmetic product [19]. The results showed that increasing the nanoemulsion concentration led to a decrease in viscosity across Formulations 1 to 3. Polyethylene glycol (PEG) exhibits specific characteristics in serum gel systems, contributing to a lower viscosity effect. This may be attributed to the viscoelastic properties of PEG [45]. According to Maharini *et al.* (2020), the interaction between Tween 80 and PEG 400 can reduce the viscosity of the formulation [46]. Excessively high viscosity can reduce the effectiveness of the nanoemulsion-based hair serum, as it may limit the mobility of active ingredients on the skin surface, thereby decreasing their penetration rate into the skin [39].

The spreadability test was conducted to evaluate the formulation's ability to disperse when applied to the skin. A lower viscosity in the nanoemulsion based hair serum formulation corresponds to a greater spreadability. This broader dispersion enhances drug absorption, allowing for faster penetration into the scalp [41]. Requirements for good spreadability of semi-solid dosage forms for topical use range from a diameter of 5-7 cm [29]. The spreadability of the three formulas was in the range of 9.54-10.36 cm. The results from Formulations 1 to 3 confirmed that as viscosity decreased, spreadability increased. There are no specific criteria regarding spreadability. Spreadability is inversely proportional to the viscosity ratio of the preparation. Additionally, the pressure applied during use also influenced spreadability, further enhancing the dispersion area of the formulation. A higher spreadability value indicates that a greater amount of the formulation can reach the application area (stratum corneum) and be evenly distributed [47]. ANOVA analysis of spreadability showed a significant difference ($p \leq 0.05$), indicating that an increase in nanoemulsion concentration led to a statistically significant effect on spreadability. However, compared to the control, Formulation K1 did not exhibit a significant difference, whereas Formulations K2 and K3 showed significant differences from the control. The adhesion test was conducted to assess the formulation's ability to adhere to the skin surface, which directly influences the effectiveness of the active ingredients in the nanoemulsion-based hair serum. Ideally, good adhesion should last for more than one second [39]. The adhesion test results for Formulations 1 to 3 indicated that all formulations met the criteria for good adhesion. However, as nanoemulsion concentration increased, adhesion duration decreased. Longer adhesion times enhance the pharmacological activity of the formulation. Statistical analysis using ANOVA showed a significant difference ($p \leq 0.05$) among all formulations, confirming that variations in nanoemulsion concentration resulted in significantly different adhesion properties.

Based on the results of the stability test, the organoleptic evaluation indicated that the nanoemulsion based hair serum containing *M. oleifera* seed oil remained in a liquid form, exhibited a yellowish-white color, and showed no phase separation, both before and after the stability test. The complete stability test data can be seen in table 3. Based on the results obtained from table 3 in general, it shows that both the pH, viscosity, spreadability and adhesiveness of nanoemulsion based hair serum preparations are not different before and after cycling test. This means that the preparation is stable during six cycles of storage. Nanoemulsion formulations enhance stability by protecting active ingredients from external exposure through their carrier system. This characteristic contributes to maintaining stability during storage [48]. Active compounds incorporated into the nanoemulsion system dissolve into the micelle core, ensuring thermodynamic stability [49]. Additionally, the appropriate combination of surfactants and co-surfactants effectively protects micelles from environmental factors, further enhancing stability [47].

Table 3: Stability test results of *M. oleifera* seed oil nanoemulsion based hair serum

S. No.	Stability test	Result	P value	Description
1	Organoleptic	Yellowish white, stable, soft texture, odorless, homogeneous	-	physically stable preparation for cycling test
2	pH test	Stable during six cycles storage	0.487	The pH of hair serum nanoemulsion stable before and after cycling test
3	Viscosity test	Stable during six cycles storage	0.126	The viscosity of nanoemulsion based hair serum was not different before and after cycling test
4	Spreadability test	Stable during six cycles storage	0.608	The spreadability of nanoemulsion based hair serum was not different before and after cycling test.
5	Adhesion test	Stable during six cycles storage	0.539	The adhesion of nanoemulsion based hair serum was not different before and after cycling test.

The pH results obtained a decrease in pH before and after the test. However, based on the results of the paired t test, there was no significant difference with a value of 0.487 ($p>0.05$). The pH values of the nanoemulsion-based hair serum remained within a safe range for topical application, even under both low and high-temperature storage conditions. The results of the viscosity test, after the test obtained data on the decrease in viscosity. The paired t test results showed a value of 0.126, meaning that there was no significant difference before and after the stability test. During storage, the viscosity of the nanoemulsion based hair serum formulation decreased. This reduction in viscosity may be attributed to storage conditions, particularly temperature fluctuations. Increased temperatures can lead to base degradation and a reduction in intermolecular attractive forces, ultimately lowering viscosity [29]. During the stability test, the spreadability of the preparation is inversely proportional to the viscosity. The results of the paired t test for spreadability both before and after the test had no significant difference, with a significance value of 0.539. In contrast, adhesion properties were directly influenced by viscosity, with higher viscosity resulting in longer adhesion time. Among the tested formulations, Formula 3 exhibited the lowest viscosity, which corresponded to a decrease in adhesion time and an increase in spreadability. Based on the results of the paired t test, the significance value is 0.608, meaning that there is no significant difference before and after the stability test.

Nanoemulsions are kinetically stable formulations. The stability of nanoemulsions is close to thermodynamic stability, this is due to the long physical stability without noticeable coalescence and flocculation [45]. Emulsion stability is also associated with deformation mechanisms. The deformation mechanism depends on hydrodynamic interactions, particle size, surface forces as well as colloidal forces [50]. Nanoemulsion droplets are usually small, so they are not easily deformed [51]. Nanoemulsion based hair serum formula is an oil-in-water type emulsion preparation whose characteristics are non-sticky liquid and does not leave crusts. This does not cause dandruff because it does not leave crusts on the hair skin layer. The use of Tween 80 as a surfactant and PEG 400 as a cosurfactant is used as an emulgator that is hydrophilic and lipophilic [52]. Oil droplets will exhibit substantial electrostatic potential in the absence of surfactants. This is usually attributed to the absorption of hydroxyl groups, which are more favorable at the oil-water interface [37].

Numerous commercial hair serums currently incorporate natural oils such as argan, coconut, or jojoba oil, formulated either conventionally or through advanced delivery systems like nanoemulsions. These formulations typically aim to improve the bioavailability and penetration of active ingredients. In contrast, the nanoemulsion developed in this study utilizes *Moringa oleifera* seed oil-an underexplored botanical source with high antioxidant activity. The formulation demonstrates desirable physicochemical characteristics, including a droplet size range 200 nm and appropriate viscosity for topical use. Its distinct composition positions it as a potentially competitive alternative in the growing market for natural and functional hair care products. Previous studies have demonstrated the potential of nanoemulsion-based delivery systems in enhancing the performance of botanical hair care products. For instance, Dhariwala *et al.* (2019) showed that green tea extract nanoemulsions exhibited high bioactivity and stability when formulated as hair serums [53]. In comparison, the present formulation introduces *Moringa oleifera* seed oil as a novel active ingredient with a rich profile of essential fatty acids, tocopherols, and phytosterols, known to support hair and scalp health. Furthermore, the formulation demonstrated physical stability over a six cycle highlighting its commercial viability as a plant-based nanoemulsion based hair serum.

CONCLUSION

Variations in nanoemulsion concentration influenced its physical properties. A higher concentration of *M. oleifera* seed oil nanoemulsion leads to decreases in pH, viscosity, and adhesion, while spreadability increases. Formula K2 was selected as the optimal formulation, characterized by a liquid form, yellowish-white color, homogeneity, a pH value of 6.27±0.07, a viscosity of 208.98±5.35 cPs, an adhesion time of 42.40±3.05 sec, and a spreadability of 10.06±0.19 cm. This formulation demonstrates potential as an effective hair growth agent by offering reduced stickiness and greasiness, good spreading properties, and the ability to achieve maximum therapeutic effect with lower active ingredient concentrations, making it highly promising for future topical hair growth applications. Furthermore, the nanoemulsion-based hair serum met the stability requirements both before and after the cycling test. While the current study focuses on formulation optimization and stability, in vivo validation is essential to confirm the product's functional efficacy.

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Conceptualization: Mutmainah, Akhmad Kharis Nugroho, Triana Hertiani, Adhyatmika. Methodology: Mutmainah, Akhmad Kharis Nugroho, Triana Hertiani, Adhyatmika. Analysis: Mutmainah. Investigation: Mutmainah, Akhmad Kharis Nugroho, Triana Hertiani, Adhyatmika. Resources: Mutmainah, Akhmad Kharis Nugroho, Triana Hertiani, Adhyatmika. Data curation: Mutmainah. Writing Original Draft preparation: Mutmainah. Review and editing: Akhmad Kharis Nugroho, Triana Hertiani, Adhyatmika. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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