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Innovative Gel Formulation Incorporating *Vernonia amygdalina* Leaf Extract Nanoparticles: Physical Evaluation and Moisturizing Potential

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Abstract: *Vernonia amygdalina* exhibits relatively weak antioxidant activity with an IC_{50} value greater than 200 ppm. This study aimed to evaluate the formulation of *Vernonia amygdalina* leaf extract nanoparticle (NEDA) to enhance the absorption and stability of its active compounds in a gel preparation intended for use as a moisturizer. The NEDA was formulated using chitosan and NaTPP with the following compositions: F1 (1:10:1), F2 (1:15:1), and F3 (1:20:1). The concentrations of NEDA incorporated into the gel formulations were prepared in four variations: G0 (0%), G2 (5%), G2 (10%), and G3 (15%) using nanoparticles from the optimized F3 formula, with Carbopol employed as the gelling agent. The evaluation conducted on the formulations included particle size, particle morphology, organoleptic properties, homogeneity, viscosity, pH, spreadability, adhesiveness, moisturizing effect, and stability. The result showed that the particle size of NEDA F1, F2, F3 were 343±26.87 nm, 121.5±4.94 nm, and 81.5±0.70 nm, respectively. Transmision Electron Microscopy (TEM) revealed that the nanopartcile exhibited spherical morphology. The antioxidant activity evaluated using the DPPH method showed the IC_{50} of 194±0.86 ppm for the crude extract (EDA), whereas NEDA F3 demonstrated a stronger antioxidant activity with an IC_{50} value of 124.5±0.67 ppm. The organoleptic testing indicated that the NEDA gel formulations G0, G1, and G2 were clear, while G3 appeared slightly cloudy; all formulas were homogeneous. The viscosity testing showed that the G0-G3 formulations ranged between 35000-49000 cP. The pH values of the gels were within the normal skin pH range (4,1-5,1). The spreadability and adhesiveness tests revealed values ranging from 4,5-5,2 cm an 3,7-4,8 seconds, respectively. The moisturizing effect evaluation performed on G2 demonstrated an increase in skin moisture from 40% to 47% after 2 hours of application. In conclusion, the NEDA gel formula G2 was determined to be the most optimal. No significant differences were observed in the physical evaluation parameters during 28 days of storage (P=0,220), indicating good formulation stability.

Keywords: antioxidant; gel; IC^{50}; moisturizer; nanoparticle

1. INTRODUCTION

Premature aging has become a serious concern, particularly among women. Aging is a complex biological process involving multiple mechanism, in which celluler damage and repair occur alternately at varying rates and durations [1]. Oxidative stress caused by free radicals plays a significant role in accelerating the aging process, leading to skin damage and loss of elasticity.

The *Vernonia amygdalina* is known to possess antioxidant activity; however, its activity remains relatively weak, with an IC_{50} value greater than 200 ppm [2]. This plant contains various secondary metabolites, including flavonoids, alkaloids, tannins, terpenes, saponins, phenolic compounds, and sesquiterpenoids [3]. The characteristic bitter taste of *Vernonia amygdalina* is primary attributed to the presence of sesquiterpenoids, which belong to the terpenoid group, as well as other compounds such as flavonoids [4].

Antioxidants are essential subtances required by the body to neutralize free radicals and protect normal cell, proteins, and lipids from oxidative damage [5]. However, the absorption of *Vernonia amygdalina* extract is still considered low; therefore, particle size reduction to the nanoscale is

necessary to enhance its bioavailability and penetration [6]. Previous studies reported that liquid soap formulations containing *Vernonia amygdalina* leaf extract exhibited IC_{50} values of 772,087 ppm (F1), 565,718 ppm (F2), and 261, 305 ppm (F3) [2]. Additionally, antioxidant activity testing using 70% ethanol extract showed an IC_{50} value of 371,98 ppm (FI), while the 96% ethanol extract demonstrated an IC_{50} value 557,058 ppm (F2) [7]. Other studies alse reported an IC_{50} value of 557,058 ppm from *Vernonia amygdalina* leaf extract [8].

Nanoparticle-based delivery systems have been widely reported to enhance the pharmacological performance of bioactive compounds through multiple physicochemical and biological mechanism. The improvement in activity is primarily attributed to the nanoscale size, which significantly increases the surface area to volume ratio, thereby enhancing the dissolution rate and the availability of active molecules for interaction with biological targets. According to the Noyes-Whitney principle, a reduction in particle size leads to an increase in dissolution velocity, which consequently improves the apparent bioavailability of poorly soluble phytoconstituents [9]. Nanoparticles are particles with sizes ranging from 1 to 100 nanometers, although some approaches suggest that the optimal size range between 200 and 400 nm. [9,10].

In addition to improving pharmacological performance, nanoparticle systems significantly enhance the physicochemical stability of formulations. The formation of a crosslinked polymeric network in chitosan-NaTPP nanoparticle prevents aggregation and phase separation, resulting in a more stable colloidal system. This improved stability is particularly important when nanoparticle are incorporated into semisolid dosage forms such as gels, where uniform distribution and prevention of sedimentation are critical for maintaining dose consistency and reproducibility [11].

The gel is a semisolid system composed of suspensions of small inorganic particles or large organic molecules dispersed in a liquid medium [12]. Moisturizers are skincare product designed to alleviate skin dryness by softening the skin, filling pores, and reducing surface friction. They function by preventing transepidermal water loss and maintaining skin hydration through the formation of a lipid layer on the skin surface [13].

In this study, Carbopol was used as gelling agent, white Triethanolamine (TEA) served as a base to neutralize the gel pH and enhance Carbopol stability. Carbopol was selected due to its excellent dispersibility in water at low concentration and its ability to produce adequate viscosity [14]. Based on the above background, this study aims to evaluate the formulation of *Vernonia amygdalina* leaf extract nanoparticle incorporated into gel dosage form as a moisturizer.

2. MATERIALS AND METHODS

2.1. Materials

The equipment used in this study included an analytical balance, graduated cylinders, beakers, watch glasses, glassware, glass stirring rods, a Brookfield viscometer, a pH meter, Particle Size Analyzer (PSA), homogenizer, and other standard laboratory instruments.

The materials used in this study consisted of *Vernonia amygdalina* leaf, chitosan, sodium tripolyphosphate (NaTPP), glycerin, carbopol, triethanolamine (TEA), methyl paraben, propyl paraben, distilled water, and deionized water.

2.2. Methods

2.1.1. Preparation of *Vernonia amygdalina* Leaf Samples

The fresh *Vernonia amygdalina* leaves were washed thoroughly under running water and drained, yielding an initial weight of 9 kg. The leaves were then air-dried until a slight brownish discoloration appeared, resulting in a dried weight of 6 kg. Subsequently, the leaf blades were separated from midribs, producing 2 kg of material. The dried leaves were futher pulverized using a blender to obtain 1 kg of fine powder, which was then sieved using an appropriate mesh size to obtain uniform simplicial powder.

2.1.2. Preparation of *Vernonia amygdalina* Leaf Extract (EDA)

The extraction of *Vernonia amygdalina* leaf powder (1 kg of dried simplicial) was carried out using the maceration method with 96% ethanol as the solvent for 3x24 hours. The solvent was replaced

every 24 hours to ensure optimal extraction. After maceration, the mixture was filtered to separate the residue from the filtrate. The filtrate was then concentrated using a rotary evaporator at 70°C to obtain a viscous extract [6]. The extraction yield was calculated using the following formula:

$$Yield\ (\%) = \frac{weight\ of\ extract}{weight\ of\ dried\ simplicia} x100$$

2.1.3. Preparation of *Vernonia amygdalina* Leaf Extract Nanoparticle (NEDA)

Chitosan 0,2% (w/v) solution was prepared by dissolving 0,2 g of chitosan powder in 100 mL of 1% acetic acid. The solution was stirred using a magnetic stirrer at a speed of 1000 rpm for 30 minutes until a homogeneous solution was obtained. Sodium tripolyphosphate (NaTPP) 0,1% (w/v) solution was prepared by dissolving 0,1 g of NaTPP powder in 100 mL of distilled water. The solution was stirred using a magnetic stirrer at 1000 rpm for 30 minutes to ensure complete dissolution.

Table 1. Formulation Design of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA)

Ingredients	Function	Comparison of Ratio			
		F0	F1	F2	F3
Vernonia amygdalina leaf Extract (EDA)	Active ingredient	0	1	1	1
Chitosan	Stabilizing and crosslinking agent	1	10	15	20
Sodium tripolyphosphate (NaTPP)	Crosslinking agent	1	1	1	1
Tween 80	Surfactant	0.5%	0.5%	0.5%	0.5%
Distilled water	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

The preparation of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA) was performed using the ionic gelation method. All materials were weighed according to the formulation shown in Table 1. Initially, the *Vernonia amygdalina* leaf Extract (EDA) as the active ingredient was mixed with chitosan solution, which acted as a stabilizing and crosslinking agent. The mixture was stirred using magnetic stirrer at 1500 rpm for 30 minutes until a homogeneous solution was obtained. Subsequently, NaTPP was added dropwise as a crosslinking agent, followed by continuous stirring at 1500 rpm for 30 minutes. In the final stage, Tween 80 was added as a surfactant, and the mixture was further stirred using a magnetic stirrer at 1500 rpm for 30 minutes. The resulting nanoparticles was then subjected to a sonication process using sonicator waterbath operating at 40 kHz and 100 W for 60 minutes at room temperature to reduce particle size and enhance nanoparticle stability [15].

2.1.4. Evaluation of *Vernonia amygdalina* Leaf Extract Nanoparticle (NEDA)

a. Particle Size and Globule Distribution Analysis

Particle size and globule size distribution of the *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA) were determined using a Particle Size Analyzer (PSA). Approximately 1 mL of the NEDA sample was placed into a cuvette, which was then positioned in the sample holder for measurement over 15 minutes to obtain particle size values and distribution curves. After each measumerement, the cuvette was throughly cleaned to remove any residual lipid contaminants [16]. Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

b. Nanoparticle Morphology Analysis

Nanoparticle morphology was examined using Transmission Electron Microscopy (TEM). The volume of 10 µL of the nanoparticle sample was dropped onto a specimen surface. The 400-mesh grid was placed on the droplet and allowed to stand for 1 minute. Excess nanoparticle solution was carefully removed using filter paper. Subsequently, 10 µL of uranyl acetate was added onto the grid as a contrasting agent and gently blotted with filter paper. After being left to dry for 30 minutes, the grid was inserted into the TEM instrument for image acquisition [15].

c. Antioxidant Activity Assay of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA) [10]

- Preparation of 50 ppm DPPH Solution
  The total of 50 mg of DPPH was dissolved in ethanol p.a and transferred into a 50 mL volumetric flask. The ethanol p.a was added up to the mark to obtain a 1000 ppm DPPH stock solution. The stock solution was subsequently diluted to 500 ppm and then further diluted to 50 ppm using ethanol p.a.
- Determination of Maximum Wavelength of DPPH Solution
  The maximum wavelength (λmax) was determined by mixing 2 mL of 50 ppm DPPH solution with 2 mL of ethanol p.a in a test tube. The absorbance was scanned over a wavelength range of 400-600 nm using the Spectrophotometer UV-Visible.
- Preparation of Vitamin C Stock Solution
  The total of 50 mg of vitamin C was dissolved in ethanol p.a and diluted to a final volume of 50 mL to obtain a stock solution with a concentration of 1000 ppm. Serial dilutions were prepared to obtain vitamin C concentration of 0,1; 0,3; 0,5; 0,7; 0,9; and 1,1 ppm.
- Preparation of *Vernonia amygdalina* leaf Extract (EDA) Solution
  The total of 100 mg *Vernonia amygdalina* leaf Extract (EDA) was dissolved in ethanol and diluted to 100 mL in a volumetric flask to obtain a stock solution of 1000 ppm. Serial dilutions were prepared to obtain concentrations of 50, 60, 90, 120, and 140 ppm.
- Preparation of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA) Solution
  The total 100 mg of NEDA from each formulation was dissolved in ethanol and diluted to 100 mL in a volumetric flask to obtain a stock solution of of 1000 ppm. Serial dilutions were prepared to obtain concentrations of 50, 60, 90, 120, and 140 ppm.
- Determination of Sample Absorbance
  Each test solution at a specific concentration was mixed with 2 mL of DPPH solution in a vial and shaken until homogeneous. The mixture was incubated for 30 minutes in the dark, after which the absorbance was measured using the Spectrophotometer UV-Visible at wavelength of 517 nm. Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.
- Data Analysis
  The absorbance values obtained were used to calculate the percentage of inhibition for each concentration of nanoparticle and extract samples. The IC_{50} value (inhibitory concentration) was determined using linear regression analysis based on the equation y = bx+a, where x represents the concentration of the test solution and y represents the percentage of antioxidant activity. Statistical analysis of the formulation characteristics was performed using ANOVA.

2.1.5. Preparation of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA) Gel

The formulation of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA) Gel is presented in Table 2.

Table 2. Formulation of *Vernonia amygdalina* leaf Extract Nanoparticle Gel (NEDA Gel)

Ingredients	Functions	Concentration (%)			
		G0	G1	G2	G3
Vernonia amygdalina leaf Extract Nanoparticle (formula F3)	Active ingredient	0	0.5	1	2
Glycerin	Humectant	15	15	15	15
Carbopol	Gelling agent	1.5	1.5	1.5	1.5
Triethanolamine (TEA)	Alkalizing agent	0.5	0.5	0.5	0.5
Methyl paraben	Antibacterial preservative	0.2	0.2	0.2	0.2
Propyl paraben	Antifungal preservative	0.18	0.18	0.18	0.18
Distilled water	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

The Carbopol (1,5%) was initially dispersed in distilled water as the first step in preparing the gel moisturizer formulation using magnetic stirrer at 500 rpm for 30 min until complete hydration was achieved. Subsequently, TEA (0,5%) was added to neutralize the Carbopol dispersion and induce gel formulation. The mixture was stirred until a homogeneous gel base was obtained under gentle stirring at 300-400 rpm for 10 min. Glycerin (15%), methyl paraben (0,2%), propyl paraben (0,18%), and NEDA at concentration 0%, 0,5%, 1%, and 2% were then incorporated into the Carbopol gel base under stirring at 300-500 rpm for 15 min. Distilled water was added to the final volume of 100%, and all components were thoroughly mixed and homogenized at 500 rpm for 10 min to obtain a stable gel moisturizer formulation [21].

2.1.6. Evaluation of *Vernonia amygdalina* leaf Extract Nanoparticle Gel (NEDA Gel)

a. Organoleptic Test

Organoleptic test of NEDA gel moisturizer was conducted under different temperature conditions. The room temperature (25-28°C) was used for general assessment, higher temperatures (30-40°C) were applied to simulate hot environmental conditions, and lower temperatures (15-20°C) were used to stimulate cold conditions. Changes in color and odor were observed and recorded [6]. Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

b. Viscosity Test

Viscosity measurements were performed using a Brookfield Viscometer. The gel sample was placed on a cone with diameter of 5 cm, and plate was applied to press the gel into a thin layer. Care was taken to avoid friction between the plate and the cone during measurement. Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

c. pH Test

The pH meter was calibrated using standar buffer solutions prior to analysis. Approximately 0,1 g of the gel sample was dissolved in 10 mL of distilled water, and the pH was measured. The acceptable pH range for topical skin preparations is 4,5-6,5 [17]. Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

d. Spreadability Test

The total of 0,5 g of gel sample was placed at the center of a graduated circular glass plate. Another circular glass plate of transparent material was placed on top of the gel, along with an additional weight to achieve a total load of 150 g. After 1 minute, the diameter of gel spread was measured. An acceptable spreadability value for gel formulations ranges from 5 to 7 cm [18]. Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results.

e. Moisturizing Effect Test

Temperature and humidity significantly influence the stability and performance of the gel moisturizer formulations. Excessively high temperatures may cause degradation of formulation components, while high humidity may promote microbial growth or induce physical changes in the gel. Therefore, moisturizing efficacy testing was conducted at room temperature (25-28°C) with an ambient relatively humidity of approximately 46-55%. The moisturizing effect was evaluated on fifteen female volunteers aged 19-40 years old with dry skin conditions, using Digital Skin Analyzer. Prior to participation, all volunteers were informed about the study procedure and signed an informed concent form indicating their willingness to participate in the moisturizing efficacy test. Prior to application, the test area was washed and dried thoroughly. The gel sampel (G0-G3) were applied to designed areas on the dorsal surface of both the left and right hands, covering an area 3x3 cm. Each measurement was performed in triplicate to ensure the reliability and reproducibility of the results. Baseline skin moisture levels from all participant were measured and recorded. Skin moisture levels were measured after 1 minute, and 2 hours, and the percentage values were recorded. Skin moisture levels were classified as dehydration (0-29%), normal (30-45%), and hydration (50-100%). Water content plays a crucial role in determining the physical properties of gel formulations; higher water content generally results in softer and more flowable gels, whereas lower water content may

produce harder and more rigid gels. An optimal water content for NEDA gel moisturizers typically ranges between 70% and 80%.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Plant Determination

The identification of the *Vernonia amygdalina* leaf was conducted at the Herbarium Laboratory, School of Life Sciences and Technology (SITH), Bandung Institute of Technology. The plant determination was performed to ensure the authenticity and taxonomic accuracy of the plant material used in this study. Based on taxonomic analysis, the plant was identified as *Vernonia amygdalina,* belonging to the family Asteraceae.

3.1.2. Preparation of *Vernonia amygdalina* leaf Extract (EDA)

In this study, total of 5000 g of *Vernonia amygdalina* leaves were collected from Remban Village. The extraction process was carried out using the maceration method. The extraction yield obtained was 10,6%, resulting in 53 g of concentrated *Vernonia amygdalina* leaf extract. This yield indicates that the maceration method using ethanol was effective in extracting the bioactive compounds from *Vernonia amygdalina* leaves.

3.1.3. Evaluation of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA)

a. Particle Size and Globule Distribution Analysis

Particle size analysis was performed to evaluate the effectiveness of the nanoparticle formulation process. The particle size of NEDA was measured for each formulation using PSA. Particle size distribution is a critical parameter influencing the stability, penetration ability, and bioavailability of nanoparticle-based formulations. The results of the particle size analysis for each NEDA formulation are presented in Tabel 3.

Table 3. Results of Particle Size Analysis NEDA

No	Formula	Particle Size (nm)	Polidispersity Index (PI)
1.	F0	106 ± 1.41	0.180 ± 0.022
2.	F1	343 ± 26.87	0.134 ± 0.020
3.	F2	121.5 ± 4.94	0.209 ± 0.155
4.	F3	81.5 ± 0.70	0.178 ± 0.096

The organoleptic observations demonstrated that the nanoparticle formulations were physically stable, showing no changes in odor, color, or appearance, and remained homogeneous throughout the storage period from day 0 to day 28.

b. Nanoparticle Morphology Analysis

Transmission Electron Microscopy (TEM) was employed to observe the morphology of the nanoparticle and to verify their particle size. The formulation selected for morphology analysis was the most stable formulation exhibiting the smallest particle size among all formulations, namely F3. The nanoparticle morphology is presented in Figure 1. The particle size distribution obtained from the measurement of 200 individual particles showed that average diameter of 78.6 ± 18.9 nm with particle sizes mainly distributed between 50-110 nm with PI 0.247.

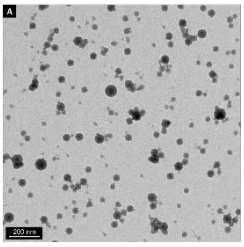


Figure 1. Nanoparticle Morphology F3 of NEDA

c. Antioxidant Activity Assay of *Vernonia amygdalina* leaf Extract Nanoparticle (NEDA)

The measurement results showed that the maximum wavelength was 517 nm, with the blank absorbance value of 0,718. The determination of this wavelength was conducted to identify the maximum absorption point and to ensure the stability of absorbance during the assay [10]. The determination of the optimum time (OT) was performed at the mid-range concentration to identify the most optimal incubation time for the reaction between the sample and DPPH. The results indicated that the optimum incubation time occurred at 30 minutes. Subsequently, a calibration curve was constructed using Vitamin C as a positive control with concentration variations of 0,1; 0,3; 0,5; 0,7; 0,9; and 1,1 ppm, from which a linear regression equation and the coefficient of determination (R^2) were obtained.

Table 4. The Results of Antioxidant Activity Test using DPPH Method

Formula	The IC_{50} value (ppm)	Antioxidant Activity category	R^2
EDA	194.5 ± 0.86	Weak	0.9047 (y=0.3026x-8.881)
NEDA F1	147.2 ± 0.66	Weak	0.9700 (y=0.4563x-17.202)
NEDA F2	128.8 ± 2.25	Middle	0.9879 (y=0.5388x-22.267)
NEDA F3	124.5 ± 0.67	Middle	0.9352 (y=0.4556x-6.7395)
Vitamin C	0.718 ± 0.0078	Very strong	3.9906 (y=59.471x+7.2671)

3.1.4. Evaluation of *Vernonia amygdalina* leaf Extract Nanoparticle Gel (NEDA Gel)

a. Organoleptic Test

Organoleptic test of the NEDA Gel moisturizer was conducted by assessing changes in textures, color, and odor [6]. The physical appearance of the prepared NEDA Gel formulations is presented below in Figure 2.



Figure 2. The NEDA Gel Preparation G0, G1, G2, and G3 (from left to right)

b. Viscosity Test

The viscosity test results of the NEDA Gel moisturizer formulation F0 (base), F1, F2, and F3 indicated that all formulations exhibited acceptable viscosity values, ranging from 30000-50000 cP. These results demonstrate that the incorporation of NEDA gel at various concentrations did not adversely affect the viscosity of the gel formulation.

c. pH Test

The pH measurements were conducted using pH meter. The acceptable pH range for gel moisturizer formulations is 4,5-6,5 [2]. The pH measurement results of the NEDA gel formulation (G0-G3) showed that all formulations exhibited an increase in pH from day 0 to day 28th (range 4,1-5,2). Among the formulations, G2 (10%) remained within the acceptable pH range (4,5-5,2) for the gel moisturizer formulations throughout the entire storage period from day 0 to day 28th.

d. Spreadability Test

The spreadability test results of NEDA gel formulation showed that formulation G2 met the acceptable spreadability criteria for gel moisturizer from day 14th to day 28th. Formula G3 also fulfilled the spreadability criteria; however this was observed only during the storage period from day 7th to day 14th with range 4,5-5,2 cm.

e. Moisturizing Effect Test

The measurements were conducted at several time intervals, namely before application (0 minute), immediately after application (1 minute), and 2 hours after application. The results of the moisturizing efficacy test of the NEDA gel using formulation G2 demonstrated that all fifteen panelist

initially exhibited dry skin conditions. After 1 minute of application, the skin moiture levels increased to the moist category (47-60%). Furthermore, after 2 hours of application, the skin remained in a moist condition, with moisture level ranging from 40-47%.

3.2. Discussion

In this study, several evaluations of the NEDA gel formulation were conducted to investigate the characterization of EDA, the enhancement of antioxidant activity following nanoparticle formation, and the incorporation of NEDA into a gel moisturizer dosage form.

The particle size analysis conducted on day 0 and day 14th demonstrated that all formulations were within the expected particle size range. Formulation F0 exhibited a decreace in particle size and tended to remain stable, whereas F1 and F2 showed more pronounced increase in particle size, indicating the occurrence of agglomeration during storage. Agglomeration refers to the merging of smaller particles into larges ones due to interparticle interactions. Meanwhile, F3 also exhibited an increase in particle size; however, the increase was relatively small and remained within the nanometer range [19]. Statistical analysis using One-Way ANOVA on nanoparticle size at day 0 revealed significant differences (p=0,012) between F0 an F1, F2, and F3. Further statistical analysis conducted on day 14th also showed significant differences (p<0,05) between F0 and F1 (p=0,021) and between F1 and F3 (p=0,022). These results indicate that the lower chitosan content used in F1 had a significant effect on the resulting nanoparticle size compared to the other formulations, consistent with the role of chitosan as a stabilizing agent capable of preventing agglomeration. Differences in particle size among formulations were presumed to be caused by variations in chitosan ratios used in each formulation.

The selection of Chitosan:NaTPP was based on preliminary optimization studies and previous reports demonstrating that this ratio provides nanoparticles with desirable physicochemical characteristics. In the ionic gelation method, positively charged chitosan of interacts electrostatically with negatively charged phosphate groups of sodium tripolyphosphate (NaTPP), leading to the formation of stable crosslinked nanoparticle matrix. Excessive NaTPP concentrations may induce over-crosslinking, resulting in larger particles size, broader particle size distribution, and particle aggregation. Conversely, insufficient NaTPP concentrations may produce inadequate crosslinking and poor nanoparticle stability. According to Kurniasari and Atun [10], the higher chitosan concentrations tend to produce the smaller particles size.

Transmission Electron Microscopy (TEM) was employed to observe nanoparticle morphology and to verify particle size. The formulation selected for morphological analysis was F3, as it demonstrated the highest stability and the smallest particle size among the formulations. TEM analysis revealed that the nanoparticles exhibited a spherical shape with sizes in the nanometer scale. The particle appeared to be relatively homogeneously dispersed; however, slight agglomeration was still observed in some areas, characterized by clusters of closely packed particles. This agglomeration is attributed to attractive forces between particles arising from high surface energy.

Antioxidant activity was analyzed using DPPH method. DPPH is a stable free radical that reacts with hydrogen-donating compounds and exhibits maximum absorbance in the range of 515-520 nm. The presence of radical scavenging compounds results in a decrease in absorbance proportional to the number of electrons donated. Data analysis was performed using One-Way ANOVA following normality and homogeneity tests, which indicated that the data were normally distributed but not homogeneous, as presented in the appendix. Post hoc analysis test revealed that nearly all formulations exhibited significant differences in antioxidant activity (p<0,05). Specifically, EDA showed a highly significant difference compared to all other formulations, and F1 differed significantly from F2, F3, and the positive control. In contrast, no significant difference was observed between F2 and F3 (p=0,201). Vitamin C (ascorbic acid), used as the positive control, exhibited a lower IC_{50} value due to its nature as a pure antioxidant capable of donating electrons to neutralize free

radicals. These findings are consistent with the study by Agustin [15], which reported an increase in antioxidant activity following nanoparticle formulation compared to EDA alone.

The enhancement of antioxidant activity is attributed to the increased surface area of nanoparticle, which intensifies and optimizes interactions between antioxidant compounds and free radicals, thereby improving radical scavenging efficiency and reducing oxidative effects [20]. Additionally, nanoparticle formulation improves the stability of antioxidant compounds through electrostatic interactions between protonated amino groups of chitosan in acidic conditions and oxygen atoms in flavonoid of polyphenol structures. In this system, flavonoids act as the primary bioactive components, while the nanoparticle system functions as a carrier that enhances stability, solubility, and bioavailability, resulting in more effective antioxidant activity compared to conventional extracts. The presence of TPP further strengthens and stabilizes these interactions.

Organoleptic evaluation of the NEDA gel formulation on day 0 showed that G0 (base) dan G1 appeared clear, whereas G2 and G3 exhibited turbidity, while maintaining similar shape and odor. After 28 days of storage, no significant organoleptic changes were observed in any formulation. The observed turbidity was attributed to the presence of small particles such as plant cells, fibers, or other insoluble components. Consequently, higher concentrations of the active ingredient resulted in increased turbidity of the formulatios [21].

Viscosity testing was conducted to evaluate the consistency and flow properties of the formulations. Excessively high viscosity is undesirable as it may hinder gel extrusion from containers and complicate redispersion. The viscosity values of the NEDA gel formulation G0, G1, G2, and G3 were within the acceptable range of 30000-50000 cP, with measured values of 49000 cP (G0), 41000 cP (G1), 37000 cP (G2), and 35000 cP (G3). Very low viscosity may lead to formulation instability and non-uniform dispersion of ingredients [21].

pH measurement were performed using pH meter, with the acceptable pH range for gel moisturizer being 4,5-6,5 [2]. The pH of gel formulation should correspond to normal skin pH (approximately 4,5-5,5) to prevent irritation or other adverse skin effects. The One-Way ANOVA test revealed significant differences among formulations. Post hoc analysis showed that F2 differed significantly from G0 and G1, but not from G3. These results indicate observed pH value were influenced by formulation components, particularly TEA, which functions to neutralize Carbopol and stabilize the gel formulation, resulting in homogeneous and stable gel system [22].

The spreadability test results demonstrated that formulation G2 met the acceptable spreadability criteria for gel formulation from day 14th to day 28th, while F3 met the criteria only form day 7th to day 14th. The One-Way ANOVA test revealed significant differences (p=0,224) among formulations, and post hoc analysis indicated that G2 differed significantly from G0 and G1, while differences with G3 were less pronounced (p=0,1020). These findings suggest that 10% concentration produces optimal and consistens spreadability in accordance with gel formulation standards. Higher concentrations of EDA, particularly in G3, tended to reduce formulation viscosity, resulting in more fluid gel.

The moisturizing efficacy test aimed to evaluate the ability of the formulations to increase and maintain skin hydration after application. Measurements were performed using Digital Skin Analyzer at several time intervals; before application, immediately after application (1 minute), and 2 hours after application. Higher and more stable moisture value over time indicate superior moisturizing performance. Skin moisture levels above 40% are classified as hydrated, while levels below 36% indicate dry skin, and values below 30% are considered very dry [14]. The moisturizing efficacy results for formulation G2 showed that all fifteen panelists initially exhibited dry skin conditions. After 1 minute of application, skin moisture levels increased to 47-60%, indicating a hydrated condition. After 2 hours, skin moisture levels remained within the hydrated range at 40-47%. The ANOVA test revealed significant difference among formulations (p=0,098). These findings are consist with previous studies on nanoparticle-based topical gel formulations which reported that

the incorporation of nanoparticles into hydrogel systems can contribute to improved distribution of active compound throughout the gel matrix and enhance formulation stability [23,24]. Chitosan based nanoparticles, in particular, have been widely investigated as carriers for natural bioactive compounds due to their favorable physicochemical properties, biocompatibility, and ability to form stable dispersions within semi solid formulations [25,26]. In addition, the moisturizing effect observed in formulation G2 may be attributed not only to the presence of the nanoparticle systems but also to the synergistic effect of gel matrix components, particularly Carbopol and Glycerin. Previous studies have shown that Carbopol-based hydrogels possess excellent water-retention capacity, while gylcerin function as a humectant that attracts and retains moisture on the skin surface, thereby improving skin hydration [27].

Carbopol is a widely used gelling agent due to its high swelling capacity and ability to form clear and stable hydrogel systems at relatively low concentration. In this study, Carbopol was used at an optimized concentration of 1,5%, which was selected to achieve an appropriate balance between viscosity, spreadability, and ease of applications. The concentration of Carbopol also significantly influences the overall performance of the formulation. An optimal concentration ensures proper gel network formation after neutralization with TEA, resulting in a stable three-dimensional polymeric structure capable of entrapping bot aqueous phase and nanoparticle dispersion. This structure contributes to desirable physicochemical properties such as homogeneity, non-grittiness, and improved physical stability against phase separation [28].

4. CONCLUSION

The characteristics of the gel moisturizer formulated with *Vernonia amygdalina* leaf extract nanoparticle as the active ingredient indicated good physicochemical properties, uniform distribution, and suitability for topical application. The evaluation of gel formulation containing NEDA, based on organoleptic properties, viscosity, pH, spreadability, moisturizing effect, and stability, showed that all formulations met acceptable quality and performance standards. The effect of varying concentrations of NEDA indicated that G2 gel formulation exhibited the best overall performance among all evaluated formulations.

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