



Formulation, Physical Evaluation, and Nanoparticle Characterization of Anti-Hyperpigmentation Nano-Essence from Grape Seed Extract

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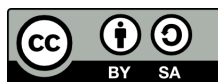
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HIGHLIGHTS

- ❖ Ethanolic grape seed extract can be formulated into an anti-hyperpigmentation nano-essence.
- ❖ The ethanolic extract of grape seeds can be formulated into an anti-hyperpigmentation nano-essence
- ❖ Grape seed extract nano-essence is physically stable and does not cause skin irritation
- ❖ This discovery provides an overview of the development of natural ingredients in collaboration with nanotechnology



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ABSTRACT

Hyperpigmentation arises from excess melanin production due to UV radiation. While conventional tyrosinase inhibitors risk long-term side effects such as hepatocarcinogenicity, proanthocyanidins from grape seed extract offer a natural, safe alternative. This study formulated grape seed extract into a topical nano-essence and evaluated its characteristics, stability, and in vivo safety. Grape seed extract obtained via sonication yielded a proanthocyanidin content of 41.483 ± 0.55 mgCE/g. To stabilize the system, formulation trials varied the ratios of propylene glycol and glycerin acting as cosurfactants, identifying F1 as the optimal formula. Nanoparticle profiling of F1 showed a Z-average of 318.4 ± 10.05 nm, a Polydispersity Index (PDI) of 0.627, and a zeta potential of -12.33 ± 0.5 mV. While these zeta potential and PDI values indicate a theoretical tendency toward microscopic agglomeration, short-term room-temperature stability testing confirmed macroscopic physical stability with no visible separation. Furthermore, safety and hedonic evaluations on 10 volunteers over two weeks demonstrated 100% absence of irritation, with 80% of subjects reporting high comfort and fast absorption. The developed nano-essence shows promising potential as a safe, cosmetically acceptable antihyperpigmentation alternative, pending further optimization to improve long-term colloidal stability.

INTRODUCTION

Hyperpigmentation is a condition in which melanin produced by the skin as a defense mechanism against ultraviolet radiation is excessively produced and eventually accumulates, causing darkening in certain areas of the skin. According to Goenka & Toussaint (2020), the hyperpigmentation mechanism is catalyzed by the enzyme tyrosinase, which catalyzes two reactions in melanin formation: the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone, which accumulation causes hyperpigmentation. According to Kong (2020), melanin production can be suppressed by inhibiting dopaquinone production using antioxidants or tyrosinase enzyme inhibitors.

Tyrosinase enzyme inhibitors refer to agents or compounds that can disrupt melanin formation by inhibiting tyrosinase activity. Commonly used tyrosinase inhibitors include hydroquinone, kojic acid, azelaic acid, and mercury. However, these compounds have harmful side effects. For example, kojic acid has hepatocarcinogenic side effects at high concentrations (Safithri, 2024). Besides these tyrosinase inhibitors, other natural substances have potential as tyrosinase inhibitors, including proanthocyanidins.

Proanthocyanidin is a type of polyphenol with various biological benefits, such as antioxidant, antimicrobial, antidiabetic, and anticancer activities (Rauf et al., 2019). In the study by Chai et al. (2017), proanthocyanidin demonstrated antityrosinase activity with an IC_{50} value of 27.1 ± 3.1 $\mu\text{g/mL}$. This value indicates tyrosinase inhibitory activity, although its IC_{50} was slightly higher than that of kojic acid (19.85 $\mu\text{g/mL}$), indicating a lower inhibitory potency under the respective assay conditions (Zilles et al., 2022). One natural source of proanthocyanidin is grape seeds.

Grape seeds (*Vitis vinifera* L.) are a major waste product of the beverage industry and contain proanthocyanidins as the main polyphenols. This makes them potential tyrosinase inhibitors. Previous research has shown that grape seed extract has antioxidant activity ($IC_{50} = 16.69$ $\mu\text{g/mL}$) and antityrosinase activity ($IC_{50} = 501.10$ $\mu\text{g/mL}$) (Habib et al., 2022). Proanthocyanidin content in grape seed extract is still rarely used for tyrosinase inhibition, making it a suitable research topic and a candidate for formulation into a dosage form.

To address hyperpigmentation, an appropriate dosage form is needed so that the active substance can be well absorbed into the skin layers. In this study, the chosen formulation concept is a "nano-essence," a lightweight, highly concentrated topical liquid vehicle that integrates nanoscale delivery systems to encapsulate active ingredients. Proanthocyanidin derived from grape seed extract is stable and non-volatile, making it suitable for formulation as a nano-essence; the preparation not only leverages the ultra-small nanoparticle size to maximize stratum corneum penetration and bioavailability but also provides a cosmetically elegant, non-sticky application with long-lasting hydration effects on the skin. However, maintaining the physical stability of such nanoscale dispersion in a liquid essence base presents a significant challenge. Therefore, propylene glycol and glycerin are incorporated as co-surfactants. Thus, the primary objective of this study is to formulate grape seed extract into a nano-essence and determine the effect of varying the ratios of propylene glycol and glycerin as co-surfactants on the physical characteristics, stability, and in vivo safety of the formulation. This study is expected to strengthen the science regarding the potential of grape seeds as an antihyperpigmentation agent while supporting environmental conservation by reducing industrial waste.

MATERIALS AND METHODS

Materials and equipments

The materials used included dried grape seeds, 70% ethanol, 96% ethanol, pro analysis (p.a.) ethanol, chloroform, concentrated H_2SO_4 , concentrated HCl (Smart Lab, Indonesia), catechin (Nitra Kimia,

Indonesia), vanillin (PT. Kimia Jaya Abadi, Indonesia), magnesium powder, amyl alcohol, 1% FeCl₃, Mayer, Dragendorff, Bouchardat reagents, Folin-Ciocalteu, Fehling A and B, chitosan (Lab Store, Indonesia), 1% acetic acid, myristic acid, sodium tripolyphosphate, PEG-40 hydrogenated castor oil, propylene glycol, Tween 80, glycerin, xanthan gum, phenoxyethanol (Raja Kimia, Indonesia), and distilled water.

The tools used in this research are a mortar, stamper, analytical balance (OHAUS), test tube (IWAKI), test tube rack, volumetric flask (IWAKI), oven, glass stirrer, magnetic stirrer, hot plate, sonicator, spatula, measuring cup (IWAKI), beaker (PYREX), cuvette, waterbath, Uv-Vis spectrophotometer (Thermo Scientific), pH meter F-71 (Horiba), micropipette (Thermo Scientific), object glass, and particle size analyzer (Horiba Sz 100z).

Methods

Preparation of grape seed simplicia

The dried grape seeds used in this study are Bali grape seeds sourced from industrial waste and purchased from an online marketplace under the store name *biji_anggur_kering*. The grape seeds were cleaned of impurities and air-dried at room temperature. Once dried, the grape seeds were ground using a grinder and stored in an aluminum foil bag at 25°C to ensure their stability.

Grape seed extraction

Grape seed simplicia were soaked in 47% ethanol (1:5) until fully submerged. Then, the mixture was sonicated for 53 minutes at 60°C and 50 kHz. Filtration was carried out using a Buchner funnel, and the residue was re-extracted with ethanol (1:5) until the solution became less concentrated. The sonicated extract was evaporated using a rotary evaporator at 50°C until thickened, then continued in a water bath at 60°C and in an oven at 50°C until a slightly viscous dry extract remained (Thilakarathna et al., 2022).

Determination of ethanol soluble extract content

A total of 5 grams of grape seed simplicia (W_0) was added to 100 mL of 96% ethanol in a stoppered bottle. The mixture was shaken periodically for the first 6 hours and left to stand for 24 hours at 25°C. The solution was then filtered, and the filtrate was evaporated to dryness on a preheated evaporating dish until a constant weight (W_1) was obtained (Kementerian Kesehatan Republik Indonesia, 2017). The ethanol soluble extract content was calculated using the equation:

$$\text{Ethanol soluble extract content} = \frac{W_1}{W_0} \times 100\%$$

Equation 1. Ethanol soluble extract content equation

Phytochemical screening

Phytochemical screening was conducted through four tests using simplicia and extract samples:

1. **Flavonoid Test:** 0.1 mg magnesium powder, 0.4 mL amyl alcohol, and 4 mL concentrated hydrochloric acid were added to 100 mg of sample. The mixture was shaken until a color change to red, yellow, or orange occurred, indicating a positive result for flavonoids (Sopianti & Sary, 2018).
2. **Tannin Test:** 100 mg of sample was extracted with 10 mL hot water and filtered. Five drops of 10% FeCl₃ were added to the filtrate. A black or blue color indicated the presence of tannins (Sopianti & Sary, 2018).
3. **Alkaloid Test:** 5 grams of sample were extracted with ammoniacal chloroform (9:1) and filtered. The filtrate was treated with H₂SO₄ and shaken. The upper layer was separated and divided into

three parts for addition of different reagents: Bouchardat, Mayer, and Dragendorff. Brown, white, and reddish-brown precipitates in the respective tests indicate alkaloids (Endarini, 2016).

4. **Polyphenol Test:** 1 gram of sample was extracted with 10 mL of hot distilled water and filtered. 5 mL of Folin-Ciocalteu reagent was added to the filtrate. A dark blue color indicated the presence of polyphenols (Sopianti & Sary, 2018).

Determination of total proanthocyanidin in grape seed extract

The determination of total proanthocyanidin was done with minimal light exposure, referring to Safitri et al. (2024) with slight modifications as follows:

1. **Solution preparation:** 10 mg of catechin was dissolved in 10 mL of p.a. ethanol (1000 ppm) as the standard stock solution. 5 grams of vanillin was dissolved in p.a. ethanol until completely dissolved and made up to 50 mL (10% Vanillin).
2. **Maximum wavelength determination:** A negative control was prepared in a 25 mL volumetric flask using 1 mL of p.a. ethanol and 5 mL of 10% vanillin, left to stand for approximately 3 minutes. Then, 10 mL of concentrated HCl was added, and the volume was made up with p.a. ethanol. The maximum absorbance of the blank was measured in the range of 400–550 nm. p.a. ethanol was used as the blank.
3. **Catechin calibration curve preparation:** Dilute the catechin stock solution to 35, 30, 25, 20, 15, and 10 ppm by pipetting into 10 mL volumetric flasks. Each was added with 2 mL of 10% vanillin, shaken, and left for about 3 minutes. Then, 4 mL of concentrated HCl was added, and the volume was made up with p.a. ethanol. The mixture was incubated for 20 minutes at 25°C, and its absorbance was measured.
4. **Determination of total proanthocyanidin in grape seed extract:** The stock extract solution was prepared by dissolving 25 mg (W) of grape seed extract in 25 mL (V) of p.a. ethanol (1000 ppm). The extract stock solution was diluted to concentrations of 350, 300, 250, 200, 150, and 100 ppm by pipetting into 10 mL volumetric flasks. Each was added with 2 mL of 10% vanillin, shaken, and left standing for about 3 minutes. Then, 4 mL of concentrated HCl was added, the volume was made up with p.a. ethanol, and mixed well. After 20 minutes of incubation at 25°C, absorbance was measured. The total proanthocyanidin content (C) in the extract was calculated using the previously established catechin calibration curve. Proanthocyanidin Content (mg CE/g extract) was calculated using the formula:

$$\text{Proanthocyanidin content (mgCE/g extract)} = \frac{C \times V \times 10^{-3} \times \text{Dilution factor}}{W}$$

Equation 2. Proanthocyanidin content equation

Nanoencapsulation of grape seed ethanol extract

The formula and procedure for nanoencapsulation were performed using the ionic gelation method adapted from Fitri et al. (2021). A 0.2% chitosan solution was prepared in 1% acetic acid. The grape seed ethanol extract was dissolved in 10% myristic acid (isopropyl myristate solvent) and mixed with 0.1% sodium tripolyphosphate (using distilled water as solvent). This mixture was then poured into the 0.2% chitosan solution, which had been mixed with Tween 80. The combined mixture was stirred with a magnetic stirrer at 800 rpm for 1 hour. **Table 1** shows the nanoencapsulation formula.

Table 1. Grape seed ethanol extract nanoencapsulation formula

Component	Formula (%)
Grape seed extract	5
Chitosan 0.2%	40
Sodium tripolyphosphate 0.1%	20
Tween 80	0.5
Myristic acid 10%	10
Distilled water	ad. 100

Formulation of grape seed ethanol extract nano-essence

The essence base was prepared by dissolving xanthan gum in hot water in a mortar and grinding it until it swelled. Propylene glycol, glycerin, and phenoxyethanol were added to the mortar and ground until evenly mixed. In another mortar, PEG-40 hydrogenated castor oil was ground and mixed into the essence base, stirred until uniform. Distilled water was then added to the essence base and stirred until homogeneous. The nanoencapsulated grape seed extract was then added into the mortar containing the essence base, ground until well mixed, and sonicated for 5 minutes at room temperature (Ningrum et al., 2024). The nano-essence formula is shown in **Table 2**.

Table 2. Grape seed ethanol extract nano-essence formula

Component	Formula (%)		
	F1	F2	F3
Grape seed extract nanoencapsulation	10	10	10
PEG-40 HCO	0.2	0.2	0.2
Propylene glycol	11.5	15	1
Glycerin	8.25	1	30
Xanthan gum	0.1	0.1	0.1
Phenoxyethanol	0.5	0.5	0.5
Distilled water	ad. 100	ad. 100	ad. 100

The formulation design, including the manufacturing procedure and the specific baseline concentrations of PEG-40 HCO and xanthan gum, was adapted from a previously validated essence formulation established by Ningrum et al. (2024). In their study, this specific combination of excipients successfully produced a stable liquid vehicle. PEG-40 HCO (0.2%) served as a non-ionic solubilizer to integrate the components, while a minimal concentration of xanthan gum (0.1%) provided sufficient

structural viscosity without compromising the lightweight nature of the essence. This established matrix therefore served as a reliable foundation for encapsulating and delivering the grape seed extract nanoparticles in this study.

Propylene glycol and glycerin were specifically selected for variation due to their dual and synergistic functions as co-surfactants and humectants. As co-surfactants, they are expected to reduce interfacial tension and provide steric hindrance, thereby preventing nanoparticle agglomeration. The variation in their percentages across F1, F2, and F3 was designed to investigate their expected influence on both physical stability and cosmetic elegance. Specifically, finding the optimal ratio is crucial to balance the lightweight, penetrating properties of propylene glycol with the moisturizing benefits of glycerin, while avoiding the excessive viscosity or stickiness that high concentrations of glycerin might impart to the nano-essence.

Physical evaluation of nano-essence preparation

Physical evaluation was conducted on the three nano-essence formulas produced, including:

1. **Organoleptic test:** The form, color, odor, and texture of the nano-essence preparation were observed using the five senses.
2. **Homogeneity test:** The nano-essence preparation was applied on a microscope slide and covered with another slide, then observed visually and microscopically. The essence preparation must show a homogeneous arrangement with no visible particles (Leny et al., 2020).
3. **Spreadability test:** One drop of nano-essence was dropped in the center of a transparent glass and covered with another glass with edges limited on all four sides. The spread formed was measured by calculating the average diameter from multiple sides. The test was repeated with added weights of 50, 100, and 200 grams.
4. **pH test:** The pH of the nano-essence was measured using a calibrated pH meter. The nano-essence was poured into a beaker, the electrode was dipped into the solution, and the reading was recorded once it stabilized. The nano-essence should have a pH range of 4.5 to 6.5, which is relatively safe for the skin.
5. **Physical stability test:** The four tests above were repeated weekly over 28 days of storage at 25°C. Consistent results from the beginning to the end of testing indicated stable preparation.

Nanoparticle profile screening

The samples consisted of three nano-essence formulations that were sent to the Integrated Laboratory and Research Center Universitas Indonesia for nanoparticle profile screening. Testing was conducted using the Horiba Sz 100z Particle Size Analyzer with test parameters including Z-average, peak analysis, and zeta potential. A good nano-essence should have particle sizes below 100 nm and a zeta potential that is away from 0 mV, specifically less than -30 mV or greater than 30 mV (Az-Zahra et al., 2022).

Irritation and hedonic test

The formula with the most optimal nanoparticle profile screening results was further tested for irritation on 10 respondents. Two testing groups were created: the test group (with extract) and the control group (without extract). Testing was conducted daily on the inner upper arm using the 4-hour patch test method for two weeks. A good topical preparation should not pose a risk of causing skin irritation such as itching, burning, and redness. Additionally, respondents assessed the preparation based on acceptability and absorption speed.

All in vivo evaluations involving human volunteers (irritation and hedonic tests) were conducted in strict adherence to ethical guidelines for human research. The study protocol was thoroughly reviewed and officially approved by the Research Ethics Committee, Faculty of Health Sciences, Universitas Pelita Harapan (Approval Number: 0032/PE.KEPK-FIKes-UPH/V/2025). Before the tests, all 10 participants were fully briefed on the study's purpose, procedures, potential risks, and their right to withdraw at any time. All volunteers provided written informed consent before any topical application of the formulated nano-essence.

RESULTS

Grape seed extraction

The extraction process was performed with one repetition, referring to the principle of repeated extraction. Based on **Table 3**, the grape seed extract yield was 5.47%, and the ethanol-soluble extract yield percentage was higher than that obtained by sonication. This indicates that maceration extraction can be an option for extracting grape seed simplicia to achieve a higher yield percentage compared to the sonication method.

Table 3. Results of sonication extraction and ethanol-soluble extract content of grape seed simplicia

Extraction steps	Simplicia weight (gram)	Extract weight (gram)	Yield (%)
Sonication	1979.12	108.23	5.47
Ethanol-soluble extract content	5	0.4687	9.374

Phytochemical screening

Table 4. Phytochemical screening results of simplicia and grape seed extract

Metabolites	Reagent	Positive Indicator	Results*	
			Simplicia	Extract
Flavonoid	Mg Powder	Red, Orange	(+)	(+)
	Mayer	White Precipitate	(+)	(+)
Alkaloid	Dragendorff	Red Precipitate	(+)	(+)
	Bouchardat	Red Precipitate	(+)	(+)
Tannin	FeCl ₃	Blue-Black	(+)	(+)
Polyphenols	Folin Ciocalteu	Blue-Black	(+)	(+)

* (+): Contains the targeted metabolite

Proanthocyanidin content determination in extract

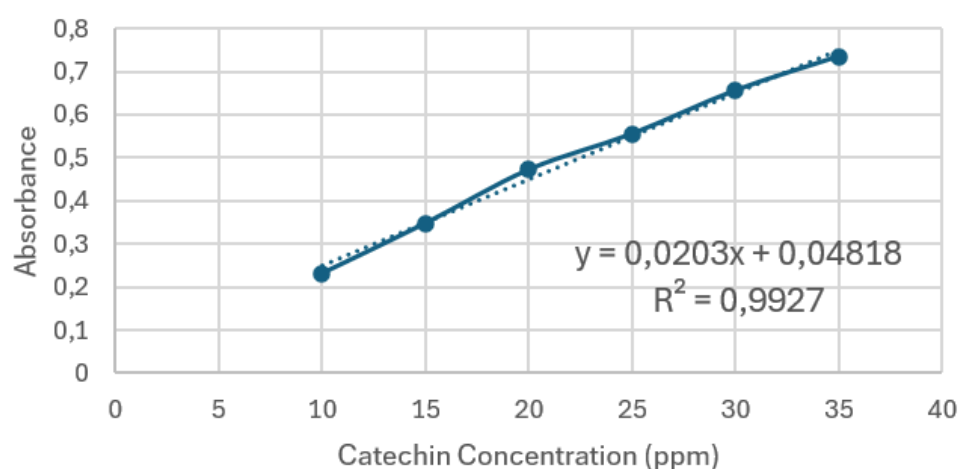


Figure 1. Standard calibration curve of pure catechin

Table 5. Absorbance and proanthocyanidin content of grape seed ethanol extract at various concentrations

Concentration of Extract (ppm)	Absorbance (Mean $\pm$ SD)	Proanthocyanidin Content (mgCE/g extract) (Mean $\pm$ SD)
100	0.099 $\pm$ 0.008	28.341 $\pm$ 4.269
150	0.161 $\pm$ 0.013	39.146 $\pm$ 4.571
200	0.203 $\pm$ 0.013	39.924 $\pm$ 3.418
250	0.275 $\pm$ 0.024	46.061 $\pm$ 4.904
300	0.331 $\pm$ 0.023	47.451 $\pm$ 3.855
350	0.377 $\pm$ 0.033	47.975 $\pm$ 4.249

Nano-essence formulation



Figure 2. Grape seed extract nano-essence

Physical stability evaluation of nano-essence

Table 6. Organoleptic evaluation of grape seed extract nano-essence

Formula	Week 1	Week 2	Week 3	Week 4
F1	The texture is slightly thick liquid, clear brownish in color, and odorless			
F2	Liquid texture, clear brownish in color, and odorless			
F3	Thick texture, clear brownish in color, and odorless			

Table 7. pH evaluation of grape seed extract nano-essence

Formula	Week 1	Week 2	Week 3	Week 4
F1	5.10	5.10	5.10	5.09
F2	5.65	5.65	5.64	5.63
F3	5.23	5.23	5.22	5.22

Table 8. Homogeneity evaluation of grape seed extract nano-essence

Formula	Week 1	Week 2	Week 3	Week 4
F1	Homogenous	Homogenous	Homogenous	Homogenous
F2	Homogenous	Homogenous	Homogenous	Homogenous
F3	Homogenous	Homogenous	Homogenous	Homogenous

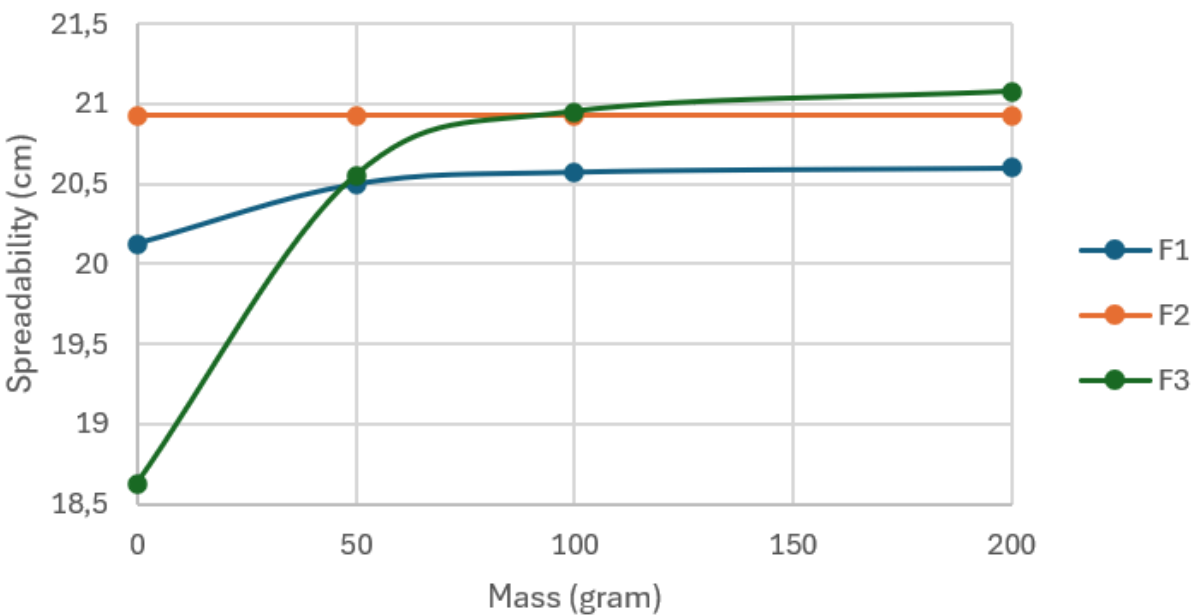


Figure 3. Spreadability graph of grape seed extract nano-essence

Nanoparticle characterization

Table 9. Nanoparticle characterization of grape seed extract nano-essence

Formula	Z Average	Peak Analysis	PDI*	Zeta Potential
F1**	318.4 ± 10.05 nm	0.8 ± 0.4 nm	0.627	- 12.23 ± 0.50 mV
F2	320.06 ± 7.41 nm	0.9 ± 0.3 nm	0.685	- 8.07 ± 1.67 mV
F3	318.57 ± 10 nm	0.9 ± 0.3 nm	0.706	- 8.07 ± 0.21 mV

*Poly Dispersity Index.

**Formula 1 is chosen as the most optimal for further irritation and hedonic testing.

Irritation and hedonic test

Table 10. Irritation and hedonic tests of nano-essence formula 1

Parameter	Essence Control	Essence Extract
Irritation	0%	0%
Acceptance	80%	80%
Quickly absorbed (< 60 seconds)	100%	100%

DISCUSSION

Sonication extraction of grape seeds yielded 5.47% extract with an ethanol-soluble extractive content of 9.374% shown in **Table 3**, confirming that the simplicia met the specific standardization parameters (Kementerian Kesehatan Republik Indonesia, 2022). Furthermore, phytochemical screening demonstrated positive indicators for all targeted secondary metabolites, including flavonoids, alkaloids, tannins, and polyphenols, as shown in **Table 4**.

Quantification of the primary tyrosinase inhibitor, proanthocyanidin, using a catechin standard curve (Equation: $y=0.0203x + 0.0418$ with R^2 of 0.9927), revealed that the grape seed ethanol extract contained a notably high average concentration of 41.483 ± 0.55 mg CE/g extract as shown in **Table 5**. This result is substantially higher than the findings reported in a previous study by Thilakarathna et al. (2022), which documented a proanthocyanidin content of 25.3 ± 1.26 mg CE/g extract. This significant difference highlights the efficacy of the sonication method used in this study and may be attributed to variations in grape variety, environmental growth conditions, and the specific solvent optimization used. This high proanthocyanidin content provides a strong rationale for advancing the extract into the topical formulation stage.

Upon formulation of the extract into nano-essences with varying co-surfactant ratios (propylene glycol and glycerin; F1–F3), physical stability evaluations over 28 days revealed consistent organoleptic characteristics. As shown in **Table 6**, all three formulas exhibited a clear brownish color with no distinctive aroma. The visually transparent appearance is a characteristic hallmark of nano-sized formulations. This aligns with Ariani & Purwanto (2021), who stated that ultra-small particle sizes minimize light scattering, resulting in a clear dispersion. However, while color and aroma remained uniform, the variations in the

co-surfactant ratios led to distinct differences in the physical texture and spreadability of the resulting nano-essences.

Formula F1 exhibited a slightly viscous texture, attributed to the balanced propylene glycol-to-glycerin ratio (11.5:8.25), which resulted in an optimal consistency. In contrast, F2 displayed the thinnest consistency among the formulations due to the dominance of propylene glycol (15:1). While fluid, this extreme thinness rendered F2 somewhat difficult to control during skin application. Conversely, F3 was the most viscous formulation, owing to its high glycerin concentration (1:30).

According to **Table 7**, the pH values of all three formulas remained relatively stable throughout the testing period, showing no significant fluctuations. All formulas exhibited skin-compatible pH levels within the safe physiological range of 4.5–6.5 (Iskandar et al., 2021). Furthermore, **Table 8** showed excellent homogeneity across all preparations. Testing revealed a uniform dispersion with no coarse particles, and this physical stability remained consistent from the beginning to the end of the evaluation period.

Unlike pH and homogeneity, the spreadability of the three formulas showed distinct flow behaviors, though all comfortably exceeded the good spreadability parameter of >7 cm shown in **Figure 3**, facilitating easy application to the skin surface (Leny et al., 2020). The variations in spreadability profiles directly corresponded to the propylene glycol and glycerin ratios. Higher propylene glycol concentrations (F2) produced a much thinner preparation that spread rapidly and achieved its maximum spread area almost immediately, with minimal applied load. In contrast, higher glycerin concentrations (F3) yielded a thicker, more viscous preparation; consequently, its spread area did not maximize instantly but rather expanded progressively as additional loads were applied during the testing.

The nanoparticle screening results in **Table 9** show conflicting parameters. The Z-average analysis indicates an average particle size of around 300 nm. Although this is within the nanometer scale (< 1000 nm), it cannot be categorized as nanoparticles, which are commonly defined as particles sized 1–100 nm (Az-Zahra et al., 2022). However, the peak analysis results for the three formulations show extremely small particle sizes, less than 1 nm. One of the peak analysis results is presented in **Figure 4**.

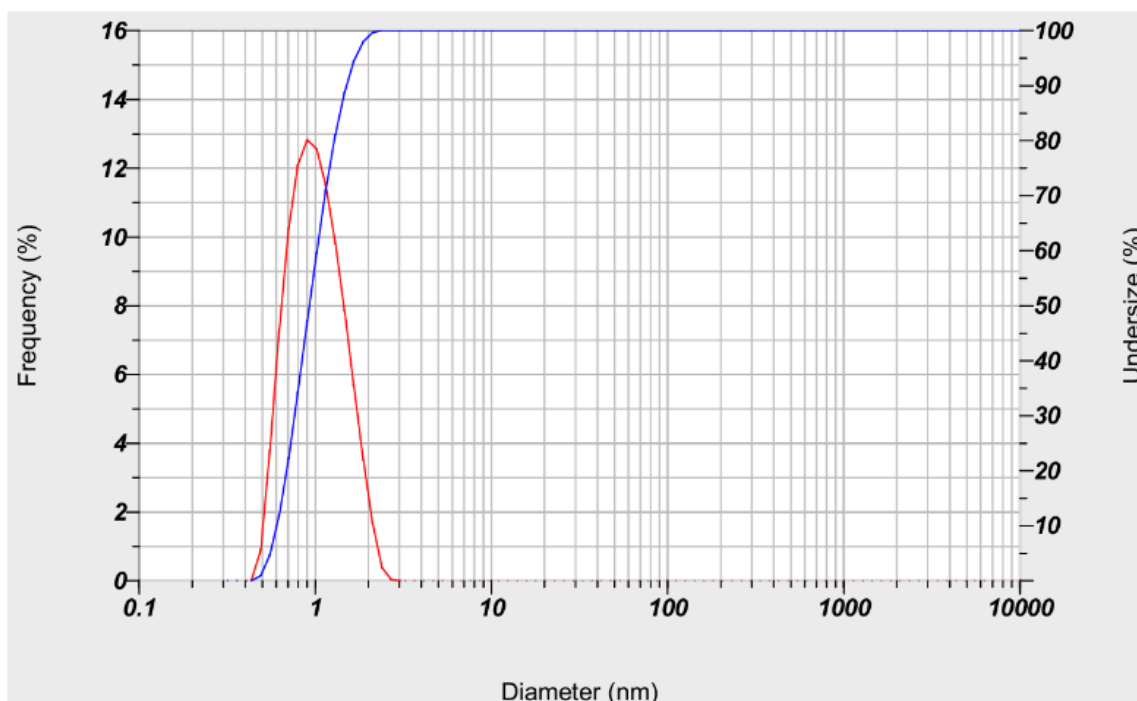


Figure 4. Peak analysis result of formula 1

Peak analysis is a method of analyzing particle size based on the frequency distribution of particles. In the peak analysis result of formula 1 shown in **Figure 4**, the red curve exhibits a peak at a diameter of less than 1 nm. However, a polymeric nanoparticle system produced by ionic gelation cannot physically exist below 1 nm, as this scale corresponds to individual molecules or ions. Therefore, this sub-nanometer peak most likely represents instrumental detection of free, unreacted small molecules in the dispersion, such as unreacted crosslinkers or high concentrations of co-surfactants (propylene glycol and glycerin), rather than the encapsulated nanoparticles themselves (Stetefeld et al., 2016).

The actual size of the formulated grape seed extract nanoparticles is accurately represented by the Z-average value of 318.4 nm. While this Z-average indicates a successful formulation in the nanometer range, it is considered relatively large. This tendency for larger average particle sizes is elucidated by the zeta potential results of the three formulas, which range from -8 to -12 mV. Nanoparticles are theoretically considered stable with strong electrostatic repulsion if their zeta potential is far from 0 mV, typically less than -30 mV or greater than 30 mV (Dipahayu & Kusumo, 2021). Although these zeta potential values suggest a theoretical tendency for agglomeration due to weak electrostatic repulsion, the macroscopic physical stability tests at room temperature showed no visible phase separation or precipitation. This indicates that the co-surfactants (propylene glycol and glycerin) likely provide sufficient viscosity or steric hindrance to suspend the nanoparticles and maintain the physical integrity of the nano-essence.

In addition to the Z-average and zeta potential, the Polydispersity Index (PDI) was evaluated to determine the size distribution homogeneity of the nanoparticles, as shown in **Table 9**. The PDI values for all formulations ranged from 0.627 to 0.706. In nanoparticle characterization, a PDI value greater than 0.5 indicates a broad size distribution, characteristic of a polydisperse system (Danaei et al., 2018). This finding perfectly corroborates the low zeta potential values previously discussed; weak electrostatic repulsion allows varying degrees of microscopic agglomeration, which broadens the particle size distribution within the dispersion. Although variations in propylene glycol and glycerin as co-surfactants did not produce drastically different overall nanoparticle profiles across the three formulas, Formula 1 was selected as the most optimal for subsequent *in vivo* testing because it exhibited the lowest PDI (0.627) and the most balanced physical consistency. However, as indicated by the electrostatic data, further optimization of the nano-essence formulation remains a critical focus for future research to significantly improve the colloidal stability of the particles.

According to **Table 10**, the irritation test of formula 1, conducted over 2 weeks with 10 respondents, showed that 100% of participants reported no signs of irritation in both the test and control groups. Additionally, 80% of respondents felt comfortable applying the nano-essence, and 100% stated that it was absorbed well and quickly, in less than 60 seconds. This indicates that the grape seed extract nano-essence is relatively safe to use, as it caused no irritation and was easily accepted by the skin.

CONCLUSION

Based on the research conducted, grape seed extract containing 41.483 ± 0.55 mg CE/g of proanthocyanidins was successfully formulated into a topical nano-essence. Formula 1 was selected as the optimal among the three formulas, with a nanoparticle Z-average of 318.4 ± 10.05 nm, a Polydispersity Index (PDI) of 0.627, and a zeta potential of -12.33 ± 0.5 mV. While the formulation demonstrated short-term macroscopic stability suitable for preliminary safety screening, the low zeta potential indicates poor electrostatic colloidal stability. Therefore, further formulation optimization and subsequent accelerated stability studies are highly required to enhance long-term colloidal stability and prevent nanoparticle agglomeration. Despite this, *in vivo* irritation and hedonic evaluations showed that 100% of

volunteers experienced no irritation, with most reporting comfort and rapid absorption. Thus, this grape seed extract nano-essence shows promising potential as a safe and cosmetically acceptable alternative for antihyperpigmentation, pending further stability refinements.

AUTHOR CONTRIBUTIONS

BNT: Conceptualization, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision/Project Administration. **AW:** Conceptualization, Formal Analysis, Investigation, Data Curation, Writing - Review & Editing. **LVP:** Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation. **KMYK:** Conceptualization, Methodology, Investigation. **CV:** Conceptualization, Methodology, Investigation. **YKH:** Conceptualization, Software/Validation, Writing - Review & Editing, Supervision/Project Administration.

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COMPETING INTERESTS

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ADDITIONAL INFORMATION

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