

Dewandaru Leaf Extract (*Eugenia uniflora* L.) as a Natural Antioxidant Serum

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Abstract

Reactive oxygen species (ROS) cause oxidative stress, damage skin architecture and collagen synthesis, and accelerate premature aging. Due to limited endogenous antioxidant defenses, external natural antioxidants such as dewandaru (*Eugenia uniflora* L.) leaf extract are needed, yet their use remains limited. This study develops and evaluates an antioxidant serum formulated from dewandaru leaf extract as an innovative anti-aging skincare solution that promotes local biodiversity. The research methodology involves a laboratory experiment that includes extracting dewandaru leaves with 96% ethanol and preparing serum formulations at two concentrations: F1 (5%) and F2 (7.5%). Physical tests (organoleptic, homogeneity, spreadability, viscosity, adhesion) showed that both formulations met SNI standards. Antioxidant activity using the DPPH method showed IC₅₀ values of 94.13 ppm for F1 (strong) and 48.20 ppm for F2 (very strong). Statistical analysis indicated a significant difference ($P < 0.005$) between formulations. In conclusion, dewandaru leaf extract was successfully formulated into a serum with antioxidant activity, with F2 (7.5%) demonstrating the highest efficacy, classified as very strong, and potential as an agent to prevent premature skin aging.

Keywords: Dewandaru leaf extract, Antioxidant, Serum, Skin Aging, DPPH.

1. INTRODUCTION

Free radicals, particularly Reactive Oxygen Species (ROS), induce oxidative stress stemming from an imbalance between cellular free radical generation (primarily in mitochondria) and antioxidant defenses. Excessive accumulation causes mitochondrial damage, reduces ATP production, diminishes membrane potential, inhibits collagen synthesis, and precipitates premature aging, leading to skin sagging and the formation of wrinkles (Xin et al., 2023). Premature skin aging is a common issue experienced by almost all segments of society. In Indonesia, 76% of women show signs of early skin aging, according to a Jakpat survey conducted on ERHA Age Corrector products. Additionally, the MEN/O/LOGY 2024 Index indicates that 42% of men also exhibit similar signs of early aging. However, most men in Indonesia are less aware of this. Symptoms of premature aging generally become visible in a person's early 30s, characterized by wrinkles and uneven skin tone. This condition can make a person look up to 6 years older than their actual age. (Rizkyah & Karimah, 2023). Therefore, strengthening the antioxidant system is the key to prevention.

The endogenous antioxidant system of the human body is limited in its capacity to neutralize free radicals, thereby necessitating external supplementation to prevent excessive

reactions (Ayu et al., 2024). Antioxidants inhibit autoxidation by breaking the free radical replication cycle, clearing peroxidation triggers, and neutralizing superoxide (O_2^-), and strengthening endogenous antioxidant defenses (Hama et al., 2025). Natural plant-based antioxidants are an ideal choice for skincare applications. To address skin problems caused by free radicals, skincare products require essential antioxidant active ingredients to combat oxidation and stimulate cell regeneration (Trasia et al., 2024). Plants are a rich source of natural antioxidants, with bioactive compounds of high antioxidant potential. Therefore, plant-based antioxidants are chosen as a safe and effective option, considering their abundant benefits and relatively minimal side effects (Trasia et al., 2024).

One of the plants that grows in Indonesia and has benefits as a traditional medicine, containing natural antioxidants, is the dewandaru plant (*Eugenia uniflora* L) (Santoso et al., 2023). The dewandaru plant, also known as Suriname Cherry, has long been used in traditional medicine. According to previous research, the ethanol extract of the dewandaru fruit, containing flavonoid compounds, shows antioxidant activity with an IC_{50} value of 53.44 ppm using the DPPH method (Santoso et al., 2022). Meanwhile, a separate study evaluating the leaves of the dewandaru plant reported significant antioxidant activity, with an IC_{50} value of 33.92 ppm determined by the ABTS method. The leaves are also rich in phenolics and flavonoids, with levels of 327.52 mg GAE/g and 35.65 mg QE/g, respectively. (Anwar et al., 2022). However, the direct interpretation that fruits have higher antioxidant content warrants reconsideration, given differences in testing methodologies. Therefore, dewandaru leaves emerge as an intriguing option owing to their potent antioxidant activity and sustainable availability, unlike seasonal fruits.

Based on the antioxidant activity potential of dewandaru leaf, prior research has demonstrated that dewandaru leaf extract can be formulated into a body lotion exhibiting potent antioxidant activity (IC_{50} 59.85 ppm) via the ABTS method (Anwar et al., 2022). However, no studies have tested face serum preparations made from dewandaru leaves. This indicates the potential to develop dewandaru leaves into various stable, easy-to-apply pharmaceutical preparations, especially serums. The advantage of serum preparations lies in their ability to contain active ingredients, which can enhance penetration into the skin and have a high safety potential because they are derived from natural ingredients (Handayani & Qamariah, 2023). This serves as motivation for researchers to conduct more in-depth studies on the benefits and antioxidant activity of dewandaru leaf extract in serum formulations.

This research aims to develop and evaluate an antioxidant serum based on dewandaru leaf extract as a local innovation that effectively prevents premature skin aging and reduces reliance on imports, while also leveraging Indonesia's biodiversity to develop modern skincare products.

2. METHOD

Research Materials

This research is a laboratory experiment. The study began with the preparation of materials, namely dewandaru leaf (*Eugenia uniflora L.*) obtained from Malang Regency. Next, extraction was performed, followed by formulation of the serum preparation, physical evaluation of the serum according to SNI standards, and antioxidant activity testing using the DPPH method. This research was conducted at the STIFAR Laboratory of the Yayasan Pharmasi Semarang for two months.

Tools and Materials

The tools used include a No. 40 mesh sieve, a blender, a maceration vessel, a measuring glass, a rotary evaporator, a UV-Vis spectrophotometer, a volumetric flask, a beaker, and a clamp stand. The materials used in this study are dewandaru leaf extract, ethano 96%, Na CMC, methyl paraben, glycerin, butylene glycol, and aquades.

Extraction

Preparation of Simplisia

To ensure the active compound content is in optimal condition, fresh dewandaru leaves are analyzed and harvested during the daytime. The dewandaru leaves (*Eugenia uniflora L.*) are cleaned with running water, then dried under direct sunlight with a black cloth cover. After drying, the leaves are ground into a powder and sifted through a No. 40 sieve. The powder that passes through the sieve is then stored in a tightly sealed container to protect it from sunlight exposure.

Extraction Preparation

Simplisia from dewandaru leaves was weighed at 500 g, then macerated with ethanol 96% to 3000 mL (1:6). Every 24 hours, the extract was filtered, and the solvent was replaced with the same volume ratio. The maceration was repeated 3 times, adding more solvent after each grinding. After obtaining the filtrate, the extract was separated from other precipitates, such as proteins, by centrifugation. Then, the extract from the dewandaru leaves was

concentrated using a rotary evaporator at 60°C until the volume of the filtrate was the same as the original ratio (Ekawati & Hariningsih, 2023).

Formulation of Dewandaru Leaf Extract Serum Preparation

The extract serum preparation of dewandaru leaves was formulated by modifying the standard formula (Pratiwi et al., 2021). The modification involved replacing Natrosol with CMC Na because CMC Na forms a network that better retains water molecules, thereby transforming the liquid into a thick gel. The formulation of Dewandaru leaf serum preparation is shown in Table 1.

Table 1 Formulation of dewandaru Leaf Serum Preparation

No	Materials	Function	Concentration(%)	
			F1	F2
1	Extract the dewandaru leaf	Antioxidant	5	7,5
2.	CMC Na	Gelling agent	1	1
3.	Methyl Paraben	Preservative	0,1	0,1
4.	Glycerin	Humectant	10	10
5.	Butylene Glycol	Solvent	3,8	3,8
6.	Aquades	Solvent	ad100%	ad100%
Final Volume			100mL	100mL

The serum manufacturing process involves combining the ingredients listed in Table 1. A 200 mL beaker is filled with sterile distilled water and then heated to 60°C-70°C. Sodium CMC Na powder is added to the beaker containing distilled water. Next, the mixture is stirred until evenly mixed, and the batter shows a consistent viscosity. Other ingredients, such as glycerin and butylene glycol, are added to the mixture while continuously stirring until homogeneous. The mixture is maintained at 60°C-70°C for 20 minutes. After that, the mixture is cooled to around 40°C, then the dewandaru leaf extract and methyl paraben are added, and the mixture is cooled further. The mixture is stirred again until it forms a homogeneous serum (Pratiwi et al., 2021). The resulting serum is then packaged in a 20 mL dark bottle. The serum will then be tested for organoleptic parameters, homogeneity, pH, spreadability, viscosity, and adhesion.

Serum Preparation Evaluation Test

Organoleptic Test

The organoleptic test was conducted through visual observation of the serum results. The observation of the preparation included smell, color, and shape of each gel formula. (Purgiyanti, 2022)

pH Test

The pH was measured using a pH meter placed on a pre-determined gel sample (Anggita & Nurhayati, 2024). The color change, according to the standard serum pH, generally falls within the pH range of 4.5 to 6.

Homogeneity Test

To assess homogeneity, the serum preparation is placed on a glass slide, after which it is examined to determine whether the constituents are evenly dispersed across the glass surface (Handayani & Qamariah, 2023).

Spreadability Test

0.5 g of a sample is placed on a glass slide and left for one minute. The sample's spread diameter is measured. Next, a load of 150 g is added and left for another minute before measuring with a constant diameter (Handayani & Qamariah, 2023).

Adhesion Test

0.25 g of a sample is placed between two glass slides on a force-testing device, then pressed for 5 minutes under a 1 kg load. After that, the load is removed, and an 80 g load is added to the device, and the serum release time is recorded (Handayani & Qamariah, 2023).

Viscosity Test

200 mL of serum is placed in a beaker, and the spindle is set to 100 rpm for 5-10 minutes. After the viscometer needle reaches the viscosity scale number, that number is recorded and multiplied by the calibration factor of the measuring device (Pratiwi et al., 2021). Viscosity was measured in this study using a Brookfield viscometer at 100 rpm, with a standard viscosity range of 230–1150 cPs.

Antioxidant Activity Test

Antioxidant activity was measured using the DPPH method. DPPH solution was prepared by measuring concentrations of 20, 40, 60, 80, and 100 µg/mL in ethanol in a

volumetric laboratory before homogenization. Next, blanko and vitamin C comparison solutions were prepared using the same method. The test solutions were prepared from those concentrations, then homogenized with a vortex and heated at 37°C for 30 minutes. Afterward, the solutions were placed in cuvettes, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition was calculated to obtain the IC₅₀ value using a linear regression formula from the percentage immersion data across all concentration solutions (Nisa et al., 2024).

Data Analysis

Each formula was replicated three times. All data from the physical evaluation of the serum preparation and antioxidant test were statistically analyzed using one-way ANOVA ($p < 0.05$) to assess the physical properties.

3. RESULT AND DISCUSSION

The determination of extract yield value is performed on the extract results of the dewandaru leaf simplisia in the form of a viscous extract. Yield is defined as the ratio between the weight of the obtained extract and the weight of the extracted simplisia powder. This yield indicates the amount of active compounds successfully extracted into the solvent. The higher the yield value, the greater the amount of active compounds contained in the solvent (Anwar et al., 2025) .

Table 2 Extraction results of dewandaru leaves

Ekstrakt	Sample Weight	Thick Extract	Yield
Dewandaru Leaf	500 gram	148,01 gram	29,60 %

Table 2 shows a yield result of 29.60%. Ethanol 96% solvent was chosen as the extraction medium due to its high polarity and rapid evaporation ability, making it very suitable for extracting chemical compounds from the dewandaru leaf (Anwar et al., 2025).

The results of the Dewandaru leaf serum formulation evaluation, including organoleptic tests, homogeneity, pH, spreadability, adhesion, and viscosity, are presented in Table 3.

Table 3 Evaluation Test of Dewandaru Leaf Extract Serum Preparation

No	Evaluation test		Result		SNI Testing Standard
			F1	F2	
1	Organoleptic	Shape	A slightly thick	A slightly thick	-
		Color	Dark Green	Deep Green	
		Smell	Typical of the dewandaru leaf	Typical of the dewandaru leaf	
2.	Homogeneity		Homogeneous	Homogeneous	Homogeneous
3.	pH		5,61 ± 0,10	4,52 ± 0,60	4,5 - 6
4.	Spreadability		6,98 ± 0,12 cm	6,45 ± 0,06 cm	5-7cm
5.	Adhesion		0,61 ± 0,34 s	1,44 ± 0,23 s	-
6.	Viscosity		777 ± 24,01 cPs	890 ± 64,66 cPs	230-1150cPs

Organoleptic

Organoleptic observations conducted in week 0 showed a dark green color in F1 and a deep green in F2, with a slightly thick texture and a distinctive smell similar to dewandaru leaves.

Uji Homogeneity

The results of the homogeneity test for the serum gel preparations from dewandaru leaf extract in each formula showed that the preparations met the homogeneity requirements, with no grains or unmixed materials observed.

pH Test

pH testing on samples F1 and F2, each repeated three times, indicates that both samples are acidic. The pH values of sample F1 were recorded sequentially as 5.71, 5.51, and 5.62 with an average of 5.61 ± 0.100 , while sample F2 showed values of 4.61, 4.53, and 4.42 with an average of 4.52 ± 0.095 . The difference is approximately 0.10 pH units. The comparison between F1 and F2 indicates that the acidity level of sample F2 is higher than that of F1. The serum preparation has met the safety requirements for application on skin with a pH of 4.5-6 (Fitria & Padua Ratu, 2022). One-way ANOVA testing showed a statistically significant difference in pH among the formulations ($P = 0.001$; $P < 0.05$).

Spreadability Test

The test results showed that both formulations met the dispersion power standards, with diameters ranging from 5 to 7 cm. (Fitria & Padua Ratu, 2022). Based on the spreadability measurement results for formulation F1 over three consecutive repetitions, the values obtained were 7.000 cm, 7.100 cm, and 6.850 cm, with an average value of 6.983 ± 0.12 cm. Meanwhile, formulation F2 produced spreadability values of 6.500 cm, 6.475 cm, and 6.375 cm, with an average of 6.450 ± 0.06 cm. Descriptively, formulation F1 shows greater spreadability than F2, with an average difference of approximately 0.558 cm. The spread diameter of the gel preparations tends to increase over time during storage, due to dilution caused by the base's inability to retain water that infiltrates the formulation (Slamet et al., 2020). One-Way ANOVA testing showed a statistically significant difference in dispersion among the formulations, with a P value of 0.003 ($P < 0.05$).

Adhesion Test

The adhesion test of the leaf preparation containing the serum extract was conducted to determine how long the serum remains attached to the skin after application. The adhesion measurement results showed that in formulation F1, the adhesion times across three consecutive repetitions were 0.41, 1.00, and 0.41 seconds, respectively. These data produced an average adhesion time of 0.61 ± 0.34 seconds. Meanwhile, formulation F2 yielded 1.21, 1.45, and 1.67 seconds across three consecutive repetitions, with an average of 1.44 ± 0.23 seconds. It can be concluded that F2 has a longer adhesive power than F1, with an average difference of 0.83 seconds. This indicates that F2 can adhere longer to the skin surface than F1. Although there is no specific standard for adhesion, it is recommended that semi-solid preparations have an adhesion time of more than 1 second (Rohmani, 2019). The One-Way ANOVA The test showed a statistically significant difference in adhesion times among the formulations ($P = 0.024$; $P < 0.05$).

Viscosity Test

The viscosity of the serum preparation was measured using a Brookfield viscometer with spindle 05 at 100 rpm. The results of the viscosity measurements demonstrated that for formulation F1, the values obtained in subsequent repetitions were 800 cPs, 780 cPs, and 752 cPs. These data yielded an average viscosity of 777.33 ± 24.01 cPs. Conversely, formulation F2 exhibited viscosity values of 912 cPs, 940 cPs, and 820 cPs, resulting in an average viscosity of 890.67 ± 64.66 cPs. Descriptively, formulation F2 possesses a higher viscosity compared to F1, with an average difference of 113.34 cPs. The viscosity measurements of both serum

formulations comply with the established standards for serum viscosity, which range from 230 to 1150 cPs. A lower viscosity is conducive to greater comfort and easier application during use (Fitria & Padua Ratu, 2022). One-way ANOVA indicated a statistically significant difference in viscosity among the formulations ($P = 0.043$; $P < 0.05$).

Antioxidant Activity Test

After the physical testing process of the preparation was completed, the antioxidant activity test was continued using the DPPH method. The antioxidant activity was assessed using the DPPH method, in which the change in the purple color intensity of DPPH was observed to be proportional to its concentration. This change was caused by the interaction between free radicals in the DPPH molecule and hydrogen atoms. Subsequently, the color change was measured with a UV-Vis spectrophotometer to determine the IC_{50} value, which reflects the free radical scavenging capacity.

Testing of each sample was performed in triplicate. The stock solution was prepared by accurately weighing 10 mg of the sample and subsequently dissolving it in 10 mL of solvent, resulting in a stock concentration of approximately 1000 ppm. From this stock solution, a series of test concentrations was prepared to achieve identical final extract concentrations (20, 40, 60, 80, and 100 μ /mL) for both formulas, calculated based on the extract percentage in each serum (5% for F1 and 7.5% for F2). Next, 0.5 mL of the sample solution with various concentrations was mixed with 0.5 mL of 0.1 mM DPPH solution, then incubated for 30 minutes at room temperature. Absorbance measurements were taken at 517 nm, with the control absorbance (without sample) recorded as 0.756. The percentage of inhibition was calculated based on the following formula.

$$\%Inhibition = \frac{[(A \text{ control} - A \text{ sample})]}{A \text{ control}} \times 100$$

The results of the antioxidant activity test of the dewandaru leaf serum is shown in Table 4. The IC_{50} value was obtained by linear regression of the sample concentration versus the percentage of inhibition.

Table 4. Antioxidant activity of dewandaru leaf serum

Formula	IC_{50} Value	Category
F1	94,13	Strong
F2	48,20	Very strong

Compounds with IC_{50} below 50 ppm are classified as very strong, 50-100 ppm as strong, 100-150 ppm as moderate, and 151-200 ppm as weak (Purgiyanti, 2022). Sample F1 with an IC_{50} of 94.13 ppm is classified as strong, while F2 with 48.20 ppm is very strong. The lower IC_{50} of F2 indicates that a higher serum extract concentration (7,5% vs 5%) provides greater free radical-scavenging capacity. The one-way ANOVA test indicates a significant difference in antioxidant activity among the formulations ($P = 0.001$; $P < 0.05$). The relationship between dewandaru leaf serum concentration and the percentage of inhibition is shown in Figure 1.

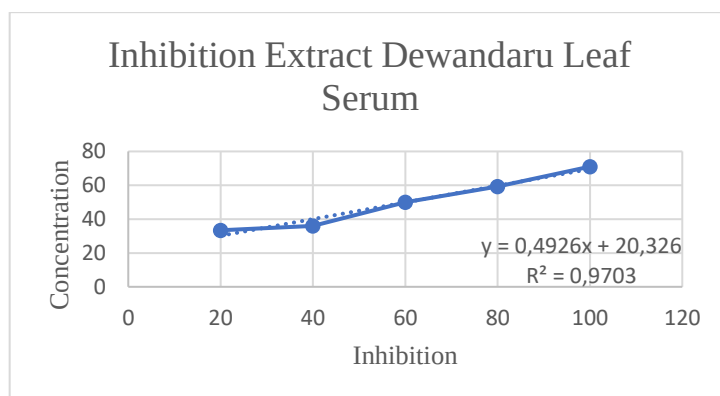


Figure 1: Inhibition Extract Dewandaru Leaf Serum

The antioxidant activity of dewandaru leaf extract serum was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method at various concentration levels. The relationship between serum concentration and percentage inhibition is presented in Figure 1. Based on the linear regression analysis shown in Figure 1, the equation $y = 0.4926x + 20.326$ was obtained with a coefficient of determination (R^2) value of 0.9703. An R^2 value close to 1 indicates a very strong positive correlation between serum concentration and inhibitory activity, where 97.03% of the variability in inhibitory activity can be explained by the variation in concentration. This suggests that the higher the concentration of dewandaru leaf extract serum, the greater the percentage of inhibition against free radicals, indicating that antioxidant activity is concentration-dependent. The results of the antioxidant activity test of dewandaru leaf serum are visually presented in Figure 2.

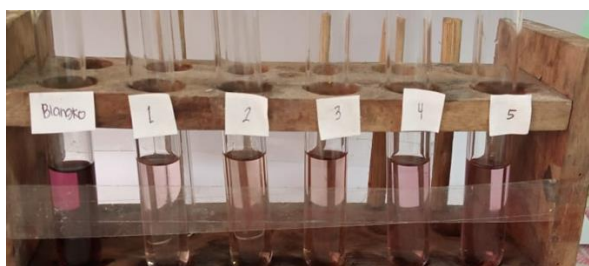


Figure 2: The results of the antioxidant activity test of the dewandaru leaf serum

Qualitative antioxidant activity testing of dewandaru leaf extract serum was conducted using the DPPH method, as evidenced by a color change from purple to pale yellow. The visual observations are shown in Figure 2. In Figure 2, tube 1 (negative control) displays a deep purple color, while tubes 2-5 with increasing serum concentrations show progressively lighter yellow intensities. The tube containing the comparison solution (Blanko) also shows a significant color change. This indicates that the dewandaru leaf extract serum has free radical-scavenging activity comparable to that of the positive control.

4. CONCLUSION

Based on the research carried out, both formulas of Dewandaru leaf extract serum meet the Indonesian National Standard (SNI) requirements for their physical tests. The antioxidant activity test on F1 at 5% showed an IC_{50} of 94.13 ppm, classified as strong, while F2 at 7.5% showed an IC_{50} of 48.20 ppm, categorized as very strong. Therefore, it can be concluded that Dewandaru leaf extract (*Eugenia uniflora L.*) can be formulated with a high antioxidant activity content. Data from the physical evaluation of the serum formulation and the antioxidant activity test, based on the One-Way ANOVA results, indicate a statistically significant difference between F1 and F2 ($P < 0.05$).

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