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Formulation and Effectiveness Test of Centella Ethanol Extract (*Centella asiatica* (L) Urb) Serum as Skin Moisturizer

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ABSTRACT

Introduction*: Indonesia has a tropical climate that tends to have high temperature and humidity, leaving skin dry and vulnerable to damage from sun exposure and pollution. One potential natural solution is the use of Centella asiatica extract, which is known to have skin hydration, regeneration, and protection effects.* ***Aims****: This study aims to develop a stable Pegagan ethanol extract-based serum formulation and evaluate its effectiveness as a skin moisturizer.* ***Methods****: Serum formulation with various concentrations of Pegagan extract (1%, 5%, and 10%), extracted using 70% ethanol through maceration method. The resulting serum was tested for physical and chemical stability, as well as its effectiveness in improving skin hydration using a skin analyzer on panelists with comparisons to anegative control (base formulation) and a positive control (brand X serum). Tests were conducted to observe the parameters of pH, viscosity, homogeneity, spreadability, adhesiveness, and effectiveness of skin hydration after product use.* ***Result****: The study showed that the serum with 1% extract concentration (F1) had the best stability, with stable viscosity and pH that is suitable for human skin (pH 5). Statistically, there were no significant changes before and after stability testing. Effectiveness testing using the skin analyser revealed a 30-50% improvement in skin hydration after 6 days of serum application, indicating that Formula F1 is effective in increasing skin moisture. Meanwhile, serums with higher extract concentrations (F2 and F3) showed decreased viscosity and instability.* ***Conclusion****: The 1% Centella asiatica extract-based serum was proven to effectively enhance skin hydration and may serve as a promising alternative skin moisturizer.*

KEYWORDS*: Centella asiatica, serum, ethanol extract, skin moisturizer, skin hydration, stability*

INTRODUCTION

The rising demand for natural-based skincare products has led to the exploration of various herbal ingredients for cosmetic formulations. One such ingredient is Centella asiatica, also known as pegagan. This plant has gained attention for its therapeutic properties, including its ability to hydrate, regenerate, and protect the skin from oxidative stress. The key bioactive compounds in pegagan—asiaticoside, madecassoside, and asiatic acid—

contribute to its ability to enhance skin function, stimulate collagen synthesis, and offer antioxidant and anti-inflammatory protection (Ahmed et al., 2019; An et al., 2012). These properties make pegagan a promising candidate for formulating skin moisturizers that not only meet aesthetic needs but also offer therapeutic benefits. Several studies have demonstrated the effectiveness of pegagan extract in improving skin hydration and epidermal barrier function. Lyko, et al. (2016) showed that a cosmetic emulsion containing 5% pegagan extract could significantly enhance skin hydration and improve the barrier function of the epidermis. This effect is attributed to the ability of asiatic acid to stimulate the synthesis of glycosaminoglycans, which help maintain moisture in the skin (Ahmed et al., 2019).

The use of pegagan ethanol extract in cosmetic formulations offers several advantages. Ethanol, as a polar solvent, efficiently extracts bioactive compounds, including triterpenoid saponins, flavonoids, and polyphenols, all of which contribute to hydration and protection against free radical damage to the skin (Yapar, 2017). However, a major challenge in formulating natural extract-based cosmetics is ensuring the stability and effective release of active ingredients. These factors are influenced by formulation parameters such as pH, viscosity, and overall stability (Pratiwi et al., 2017). Thus, a scientific approach is needed to develop pegagan-based moisturizing serums that are both effective and safe for long-term use.

While there has been extensive research on the use of pegagan, there remains a gap in studies specifically focusing on its effects on skin hydration. Most previous studies have focused on emulsion or cream formulations, whereas serum-based formulations, which have lower viscosity and greater penetration potential, have not been extensively studied. Serums offer the advantage of enhanced absorption, allowing active ingredients to reach deeper skin layers. Moreover, the stability of herbal-based products is often overlooked, yet it is crucial for ensuring the product's safety and effectiveness over time.

This study aims to develop a serum formulation based on pegagan ethanol extract and evaluate its effectiveness as a skin moisturizer. The effectiveness will be assessed using a skin analyser on panelists, allowing for a detailed evaluation of hydration levels and skin barrier function.

METHODS

Material

The materials used are distilled water (onemed®), DMDM Hydantoin (Chem-marine®), hydroxyethyl cellulose (Sigma-aldrich®), Pegagan (*Centella asiatica*),

Table 1. Design of pegagan extract serum preparation formula

Ingredient	Concentration (% w/v)				Utility
	F1	F2	F3	F4	
Dry extract of pegagan	1	5	10	-	Active substance
Hydroxyethyl sellulosa	1	1	1	1	Gelling agent
Tetrasodium EDTA	0,2	0,2	0,2	0,2	Chelating agent
Phenoxyethanol	1	1	1	1	Preservative
DMDM Hydantoin	0,5	0,5	0,5	0,5	Preservative
Aquadest	ad 100	ad 100	ad 100	Ad 100	Solvent

phenoxyethanol (Ashland®), tetrasodium EDTA (Merck®).

Sample Collection and Processing

The sample used was pegagan obtained at Lake Tanralili on the slopes of Mount Bawakaraeng, Lengkese Village, Parigi District, Gowa Regency. Fresh Pegagan herb was taken and washed in running water. Then sorted wet and chopped into small pieces. Pegagan herb that has been chopped, dried in a drying cabinet without direct sunlight until the moisture content is not more than 10% or until a constant weight is obtained with three times weighing and dry simplisia is obtained (Ahmed et al., 2019).

Extraction

Extraction was carried out by maceration method, as much as 500 g of pegagan simplisia was put into a maceration container, then 70% ethanol solvent was added in a ratio of 1:10 until the simplisia was submerged, left for 3 days and while occasionally stirring. The liquid extract that has been collected is then evaporated using a rotary vacuum evaporator until a thick extract is obtained. Then the thick extract is dried using a freeze dryer to form a dry extract. Before further use, the extract was subjected to preliminary testing to qualitatively determine the content of bioactive compounds, including triterpenoids, saponins, flavonoids, and tannins (George Joseph L., 2010).

Pegagan Serum Preparation Formulation

Weigh all ingredients according to their respective calculations (Table 1). Hydroxyethyl cellulose is developed with distilled water until it expands. Tetrasodium EDTA is dissolved in warm water. After hydroxyethyl cellulose expands, Tetrasodium EDTA is added little by little and then homogenized until homogeneous. Phenoxyethanol and DMDM hydantoin are added to the base until homogeneous. Mix the extract into the base and homogenize until homogeneous. The volume is sufficient to 50 ml (Maya et al., 2024).

Serum Preparation Evaluation

Accelerate test

One way to accelerate the evaluation of stability is to store for some period (time) at a higher, normal temperature. Evaluation of the preparation is carried out on cycles between 2 temperatures. Laboratory use, consisting of 1 cycle at 5ºC for 12 hours and 35ºC for 12 hours, was carried out for 10 cycles (Utami et al., 2023).

Organoleptic Testing

Organoleptic testing is done visually and seen directly the shape, color, smell of the preparation made.

Homogeneity Testing

Homogeneity testing is carried out by applying a gel sample to a piece of glass or other suitable transparent material, the preparation must show a homogeneous arrangement and no coarse grains are visible (Novika et al., 2024).

pH testing

pH testing is done by taking 1 gram of serum preparation sample then dissolved with 10 mL of distilled water and dipped in universal pH paper, matched the color on universal pH paper with the color table listed (Gandole, 2024).

Viscosity Testing

Viscosity measurements are made by placing a number of samples in a Brookfield® viscometer on a spindle at a certain speed. The spindle and rotation speed to be used are set, and then the tool is turned on, and the viscosity of the preparation will be read (Febriani et al., 2019).

Spreadability Testing

Scatterability testing is done by placing a 0.5 gram amount of serum between two glass plates. The glass was then given successively increasing loads (50, 100, 150, and 200 grams)(Maya et al., 2024)**.**

Adhesion Testing

Testing the adhesion of the preparation by means of serum placed on one side of the glass object with the bottom side has been paired with a rope to tie the load. Then attached to another glass object. The load used is 50 g every 5 minutes up to 250 g load. Then observe the time it takes for the load to separate the two glasses (Novika et al., 2024).

Effectiveness of the preparation on the skin with the Skin Analyzer Tool

This test has obtained an Ethical Approval Recommendation from the Health Research Ethics Commission of Muslim University of Indonesia with Number: 055/B.1/KEPK-UMI/III/2018. This test used 5 panelists. Inclusion criteria as panelists include able-bodied women, aged between 18-30 years, no history of diseases related to skin allergies and willing to become panelists by filling out a willingness form as a panelist. Exclusion criteria as panelists include women with skin disorders in the test area such as wounds

Table 2. Phytochemical screening results of pegagan extract

Test	Result
Triterpenoid	+
Saponin	+
Flavonoid	+
Tanin	+

and other skin diseases cannot be included in the study. The preparation test was conducted by applying a stable serum preparation of pegagan extract and base on the forearm of the left hand and the right hand is applied with a positive control serum preparation with an area of 3x3 cm every day. The test was carried out for 6 days, as a short-term evaluation was deemed sufficient to observe the immediate hydration effects and improvements in skin moisture. Additionally, the study aimed to assess the initial effects of serum application, which typically manifest within a few days. Effectiveness was assessed daily by observing physical changes directly and measuring skin moisture levels using a skin analyzer.

RESULTS AND DISCUSSION

This study was conducted by formulating ethanol extract of pegagan in serum preparation with variations in extract concentration to determine the formula of pegagan serum that is stable and has effectiveness as a skin moisturizer.

In this study, 1000 g of pegagan was extracted using 70% ethanol solvent by maceration method and obtained 298.0532 g thick extract with a yield of 29.80%.

Phytochemical Screening

Preliminary tests were carried out on pegagan extract using color or precipitation reactions. Phytochemical screening was conducted to detect the presence of bioactive compounds in pegagan extract (Table 2). Preliminary tests with color reaction showed that pegagan extract contains triterpenoids, saponins, flavonoids, and tannins. The presence of triterpenoids such as asiaticoside and madecassoside in pegagan extract shows the potential to increase collagen synthesis and improve epidermal barrier function (Ahmed, et al., 2019). The flavonoids and saponins detected also contribute to antioxidant and anti-inflammatory properties that support skin hydration and its protection from free radical damage (Ratz-Łyko et al, 2016).

Formulation

Organoleptic Observation

Organoleptic testing was conducted to evaluate the physical properties of serum preparations, including color, odor, and consistency. The results in Table 3 showed that serum with a concentration of 1% pegagan extract (F1) had a distinctive green color, a distinctive odor of pegagan extract, and a thick form. Other formulas (F2 and F3) with higher concentrations showed a

Table 3. Organoleptic Test Results

Formula	Inventory check	Before Accelerated Storage Conditions	After Accelerated Storage Conditions
F1	Color	Green	Green
	Smell	Typical extract	Typical extract
	Shape	Thick	A bit thick
F2	Color	Brownish green	Brownish green
	Smell	Typical extract	Typical extract
	Shape	Somewhat thick	Somewhat runny and there is extract sediment
F3	Color	Chocolate	Chocolate
	Smell	Typical extract	Typical extract
	Shape	A bit thick	A bit runny and there is extract sediment
F4	Color	Clear	Clear
	Smell	Smells distinctive	Odorless
	Shape	Thick	Thick

Table 4. Results of homogeneity testing

Formula	Before Accelerated Storage Conditions	After Accelerated Storage Conditions
F1	Homogeneous	Homogeneous
F2	Homogeneous contains extract particles	There is extract sediment
F3	Homogeneous contains extract particles	There is extract sediment
F4	Homogeneous	Homogeneous

Table 5. Results of pH measurement

Formula	Before Accelerated Storage Conditions	After Accelerated Storage Conditions
F1	5	5
F2	5	5
F3	5	5
F4	5	5

change in shape to be somewhat thinner after storage, indicating physical instability at higher extract concentrations. This observation is in line with the results of research showing that physical stability is often influenced by the concentration of active ingredients (Yapar, 2017).

Homogeneity Observation

Homogeneity testing was conducted to ensure that the mixture of active ingredients and serum base did not experience separation or sedimentation. The results Table 4 showed that Formula F1 remained homogeneous after accelerated storage. However, in Formulas F2 and F3, which had higher extract concentrations, extract sedimentation was found after the accelerated test. This instability indicates that high concentrations of centella extract may affect the homogeneity and stability of the formulation, which was also found in previous studies (Pratiwi et al., 2017).

pH Measurement

The pH measurement was conducted to ensure that the serum remained within a safe pH range and did not cause skin irritation. The measurement results in Table 5 showed that all had a stable pH of

Table 6. Viscosity test results

Formula	Average viscosity (cPs)	
	Before Accelerated Storage Conditions	After Accelerated Storage Conditions
F1	3925±1.38[a]	2250±1.93[a]
F2	2325±2.93[b]	650±2.48[c]
F3	1400±1.49[d]	250±1.24[e]
F4	4850±1.82[f]	-

Table 7. Spread power test results

Formula	Observation of Spreading Power Measurement (cm)							
	Before Accelerated Storage Conditions				After Accelerated Storage Conditions			
Load (g)	50	100	150	200	50	100	150	200
F1	5.7	6.4	7	7.2	6	6.5	7.,2	7.4
F2	5.9	6.7	7.2	7.7	5.9	6.9	7.3	7.8
F3	6.7	7.5	8.1	8.4	6.9	7.8	8	8.2
F4	4.2	5.4	5.6	5.9	4	5.1	5.3	5.7

around 5, which corresponds to the pH of human skin (pH 4.5-5.5). This supports the safety of using serum for skin application, in accordance with research results showing that an appropriate pH can help maintain skin barrier function and reduce the risk of irritation (Lyko et al., 2016).

Viscosity Measurement

Serum viscosity was measured to assess the consistency and comfort of product application. The measurement results in Table 6 showed that Formula F1 had a stable viscosity of 3925 cPs before the accelerated test and 2250 cPs after the accelerated test. The results of the t-test indicated that there was no significant difference in viscosity between the conditions before and after storage ($p>0.05$), demonstrating that the viscosity remained stable. In contrast, Formulas F2 and F3 experienced a significant decrease in viscosity ($p<0.05$), which caused the product to become thinner. This decrease in viscosity indicates physical instability at higher extract concentrations. A large decrease in viscosity can affect the comfort of application and the effectiveness of the product (Yapar, 2017).

Spread Power Measurement

Measurement of spreadability is done to determine the extent to which the serum can be spread when applied to the skin. The measurement results show Table 7 that Formula F1 has optimal spreadability, which is 5.7-7.2 cm before accelerated test and 6-7.4 cm after accelerated test. The results of the t-test indicated that there was no significant difference in spreadability between the conditions before and after storage ($p>0.05$), demonstrating that the spreadability remained stable. These results indicate that Formula F1 has good spreadability for serum, allowing easy and even application on the skin. Good spreadability is also related to the comfort of using the product and its ability to spread easily without leaving residue (Yapar, 2017).

Table 8. Adhesion test results

Formula	Observation of Adhesive Force Measurement (seconds)	
	Before Accelerated Storage Conditions	After Accelerated Storage Conditions
F1	2,59	0,52
F2	1,56	0,49
F3	1,13	0,43
F4	3,97	0,56

Adhesion Measurement

Adhesion testing was conducted to determine how long the serum can last on the skin surface after application. Formula F1 (Table 8) has relatively good adhesion, which is 2.59 seconds before the accelerated test and 0.52 seconds after the accelerated test. The results of the t-test indicated that there was no significant difference in adhesion between the conditions before and after storage ($p>0.05$), demonstrating that the adhesion remained stable. These results indicate that the serum can last well on the skin for a certain period of time, providing stable moisture to the skin during application (Yapar, 2017).

Effectiveness of Serum Preparations

Testing the effectiveness of pegagan serum skin moisture was applied to human skin and measured using a skin analyzer before and after using the serum for 6 days. The mechanism of action binds water by forming an occlusive layer. A skin analyzer is a moisture measuring instrument on the skin surface to determine the water content in the stratum corneum which can affect a person's skin moisture. The formula used in this is Formula 1 with 1% extract based on the results of stability testing after accelerated storage, the viscosity of the preparation is still within the range compared to Formula 2 and Formula 3 which experienced a decrease in viscosity that was too far after accelerated storage conditions so that the serum was rather thin.

Formula 1 was tested for humidity in humans by comparing the negative control (serum base) and positive control (moisturizing serum brand X) then measured using a skin analyzer. The measurement results are as follows in Figure 1.

Based on the results of the statistical analysis of the one-way ANOVA test, each treatment group had a significant difference between Formula 1 and positive control ($0.006 <0.05$) and Formula 1 and negative control ($0.000 <0.05$). Although in the statistical test the results obtained showed a significant difference, the test using a skin analyzer showed an increase in moisture levels which can be seen from the difference before (15,18±2,38) and after treatment (43,03±1,64) which is still within the range

of the skin analyzer measurement results parameter namely 30 - 50%. Increased

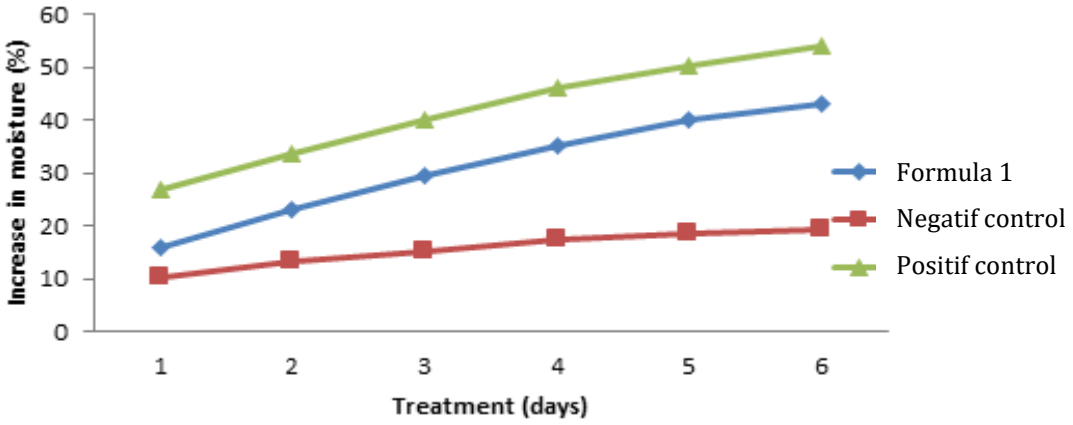

Figure 1. Graph of percentage increase in skin moisture

skin moisture by 30-50% after using the serum, which shows that Formula F1 is effective in increasing skin hydration. These results are supported by previous studies which state that pegagan can increase water content in the stratum corneum and improve skin barrier function (Ahmed et al., 2019; Lyko et al., 2016).

One limitation of this study is the small sample size of only 5 panelists. While the results provide valuable preliminary insights into the effectiveness of the serum, the small sample size restricts the ability to make statistically significant generalizations. Future studies with a larger sample size are needed to validate these findings and confirm the broader applicability of the results.

CONCLUSION

From the results of this study, a serum formula based on centella ethanol extract was obtained and has the potential as a skin moisturizer. The formula with an extract concentration of 1% (F1) showed the best results in the stability of the serum preparation. Testing using a skin analyzer showed an increase in skin moisture by 30-50% after using the serum.

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