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PHYSICAL STABILITY AND ANTIOXIDANT ACTIVITY OF ANTI-AGING SERUM FROM CENTELLA ASIATICA EXTRACT AND ROSE OIL

Purgiyanti[1]*, Rizki Febriyanti[1], Muladi Putra Mahardika[1], Susiyarti[1], Aynun Hikmah Wibasa[1]
[1]DIII Pharmacy Study Program, Harkat Negeri University, Tegal, Indonesia

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ABSTRACT

Abstract:
Centella asiatica is known as a herbaceous plant which has been widely used as a medicinal plant. One of the proven functions of this plant is as an antikeloid. One of the secondary metabolites contained in it is phenol. Rose oil also contains phenolic compounds, apart from its fragrant aroma, it has also been used as a moisturizer in cosmetic preparations. One of the benefits of this phenolic compound is as an antioxidant, in the form of a compound that can counteract free radicals. Antioxidants can affect metabolism in the body, one of which is to prevent wrinkles or aging of the skin. One of the dosage forms that can be applied as a prevention of premature aging or antiaging is serum. The purpose of this study was to make serum which would be tested for its antioxidant activity and physical stability with a storage time of 4 weeks and given different temperature variations, namely room temperature, hot and cold. Antioxidant activity test was carried out by reducing DPPH using a UV-Vis spectrophotometer. The results showed that the storage time and temperature in the stability test affected the physical test of the preparation, namely in the adhesion test, but the test results still met the requirements of the preparation and had an effect on the antioxidant activity test with a decrease in the antioxidant activity of the preparation as indicated by a greater IC50 value after storage, namely of 345.63 ppm at room temperature (25°C); 484.41 ppm in hot temperatures (40°C) and 271.64 ppm in cold temperatures (4°C) from the initial IC50 value of 98.72 ppm.

***Abstrak*:**
Pegagan dikenal sebagai salah satu tanaman berbentuk herba yang sudah banyak dimanfaatkan sebagai tanaman obat. Salah satu fungsi dari tanaman ini yang sudah terbukti adalah sebagai antikeloid. Salah satu senyawa metabolit sekunder yang terkandung di dalamnya berupa fenol. Minyak mawar juga mengandung senyawa fenol, selain karena aromanya yang wangi juga telah dimanfaatkan sebagai pelembab pada sediaan kosmetik.Senyawa fenol ini salah satu manfaatnya sebagai antioksidan, berupa senyawa yang bisa menangkal adanya radikal bebas. Antioksidan dapat mempengaruhi metabolisme dalam tubuh salah satunya adalah sebagai pencegah kerutan-kerutan atau penuaan pada kulit.Salah satu bentuk sediaan yang dapat diaplikasikan sebagai pencegah penuaan dini atau antiaging adalah serum. Tujuan dari penelitian ini adalah membuat serum yang akan diuji aktivitas antioksidan dan stabilitas fisiknya dengan waktu penyimpanan selama 4 minggu dan diberikan variasi suhu yang berbeda yaitu suhu kamar, panas serta dingin. Uji aktivitas antioksidan dilakukan dengan peredaman DPPH menggunakan instrumen spektrofotometer UV-Vis. Hasil menunjukan bahwa waktu dan suhu penyimpanan pada uji stabilitas berpengaruh terhadap uji fisik sediaan yaitu pada uji daya lekat tetapi hasil uji masih memenuhi persyaratan sediaan dan berpengaruh pada uji aktivitas antioksidan dengan terjadinya penurunan aktivitas antioksidan sediaan yang ditunjukan oleh nilai IC50 yang lebih besar setelah penyimpanan yaitu sebesar 345,63 ppm pada suhu ruang (25°C) ; 484,41 ppm pada suhu panas (40°C) dan 271,64 ppm pada suhu dingin (4°C) dari nilai IC50 awal sebesar 98,72 ppm.

Corresponding Author:
Purgiyanti,
DIII Pharmacy Study Program,
Polytechnic Harapan Bersama,
Tegal, Indonesia
Email: ipunkfalih@gmail.com

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INTRODUCTION

Indonesia is one of the largest medicinal plant user countries in the world, and has good prospects for the development of medicinal plant agribusiness. Many people have the assumption that the risks or impacts of using medicinal plants as traditional medicines are relatively safer than synthetic drugs. Adequate and correct information about medicinal plants is very necessary to achieve the optimal use of traditional medicine. With correct and adequate information, it can help the community to be more thorough in determining and using a traditional medicinal product or medicinal plant in health efforts [1].

One of the plants that has been known to be useful as medicine is the herb Pegagan. Plants that in Latin are called *Centella asiatica* It can be utilized because of its compound content which is antioxidant. The most important chemical content for these benefits is phenolic compounds[2]. We can obtain this chemical content in the form of extracts by attracting active compounds using the method of extraction, immersion or maceration with 96% ethanol organic solvent. The result of another natural ingredient that contains phenolic compounds is rose oil. This oil is obtained from red roses (*Rose damascene* P. Mill.) which is widely cultivated in Indonesia, in addition to phenolics, rose oil contains several non-polar compounds such as citronellal and graniol which can function as anti-aging or anti-aging [3].

The content of phenolic compounds contained in *Centella asiatica* extract and rose oil can be used as natural antioxidants. Antioxidant compounds are compounds that can inhibit oxidation reactions by binding to free radicals or highly reactive molecules. The antioxidant effectiveness of phenol compounds can be directly related to the structure or chemical bonds such as the degree of glycolization and the number or position of hydroxyl groups associated with carboxyl functional groups [4].

In order to get the ease of use of *Centella asiatica* extract and rose oil as anti-aging, it is necessary to make a topical preparation. That one of the topical preparations that can be used as anti-aging is *Centella asiatica* extract combined with mangosteen fruit peel in the form of a nanoemulsion[5]. Mention moisturizers that can control the occurrence of premature aging can be made from rose oil in gel preparations. Another suitable form of topical preparation is serum *spray gel* [3]. Serum itself is a slightly thick liquid product containing active ingredients that usually contain high antioxidants targeted at treating certain skin problems, for example reducing symptoms of skin aging or anti-aging [6]. This preparation was chosen because it is easy to use by applying. A good serum must meet the stability for the preparation, one of which is physical stability.

Stability is defined as the ability of a product to withstand its specified and quality limits set over periods of time of use and storage at different temperatures, i.e. at room temperature (15-30^{0}C), Hot Temperature (< 40^0 C), as well as cold temperatures (2-8)^{0}C) physical stability tests were carried out in week 0 to week 4 to see for changes in the physical properties of the preparation to ensure the identity of the strength, quality and purity of the product [7]. The stability test was carried out for 4 weeks to determine its effect on the physical stability of the serum. To determine the effect of storage time and temperature on the physical properties of serum preparations, physical stability tests need to be performed. In this study, the antioxidant activity of *Centella asiatica* extract serum preparations and rose oil was also determined after storage for 4 weeks at different temperatures as done in the physical stability test.

Previous research only used *Centella asiatica* extract as the active ingredient, so in this study, it was combined with rose oil, which functions as a moisturizer. This research is necessary because we currently

need effective, safe skincare products with minimal side effects. Furthermore, it aims to test the stability of the *Centella asiatica* extract serum formulation when combined with rose oil.

RESEARCH METHOD

Research Tools and Materials

The tools used include analytical scales, beaker glass (Pyrex), black jars for maceration, stirring rods, flannel, glass funnels (Pyrex), steam cups (Pyrex), test tubes (Pyrex), measuring flasks (Pyrex), volume pipettes, blenders, rotavapors, vials (Pyrex), measuring cups (Pyrex), micro pipettes, cuvettes, UV-Vis spectrophotometers (Ganesys 10 S). Meanwhile, the ingredients used in this study include *Centella asiatica* herb obtained from Tegal City, rose oil, aquadest, chitosan, tween 80, sacharum lactis, Aethanolum 96%, methanol, DPPH, vitamin C, FeCl3.

Research Procedure

1. Simplisia Powder Herbal *Centella asiatica*

Centella asiatica herbs are collected and cleaned from dirt, then washed with running water until clean and then dried in direct sunlight. After the simplicia is dry, it is then mashed and sifted with a mess size of 60 until a fine simplicia powder is obtained. The obtained simplicia powder is stored in a clean, dry and tightly sealed container (Extract et al., 2022).

2. Phytochemical Filtration of Phenol Compounds

Weighing the powder by 2 g, add it with aquades and heat it for about 10 minutes. The results obtained are filtered to separate the extract and the pulp. Then as many as 2 ml of samples in the test tube added 5 drops of FeCl3 will produce a blue or blackish-green color [9]. For rose oil, 5 ml of oil is taken and 5 drops of FeCl3 are added.

3. Making *Centella asiatica* Herbal Extract

A total of 150 g of *Centella asiatica* herb simplisia powder is extracted with 750 ml of 96% ethanol solvent, soaked for ± 5x24 hours while stirring occasionally, after which it is filtered to separate the pulp and filtrate. Next, the filtrate evaporates so that a thick extract is obtained. Perform an ethanol-free test on the extract by putting a small amount of the extract into the test tube, then adding 2 drops of acetic acid and 2 drops of H2SO4. Observing the change in smell, if it does not smell ethyl acetate (ester) then the extract is free of ethanol. The obtained condensed extract is weighed and stored in a tightly closed place [10].

4. Serum Manufacturing

The serum formula used uses active ingredients in the form of *Centella asiatica* extract and rose oil. Using the additives of tween 80, HPMC, and Kitosan.

Weighted HPMC is then developed in aquadest, stirred homogeneously until it produces a gel base and then let it sit for a while so that the foam disappears. Chitosan is dissolved in a warm solution of sacharum lactis then mixed with rose extracts and oils that have previously been dissolved in tween 80. Mix until homogeneous [11].

5. Testing of serum preparations

In this test, organoleptic tests, pH tests of preparations, homogeneity tests, preference tests, and adhesion tests were carried out [11].

6. Manufacture of DPPH Solution (40µg/mL)

A total of 10 mg of DPPH is dissolved with methanol in a 10 mL measuring flask, beaten until homogeneous. Then 4 mL is chopped and put into a 100 mL measuring flask, methanol is added to the limit, homogenize [12].

7. Manufacture of Serum Test Solution (50, 100, 200 and 400 ppm)

The sample preparation is weighed 100 mg, dissolved in methanol, put in a flask measuring 50 mL. The sample master solution was pipetted 0.25 each; 0,5; 1; 2 (mL) is put into a 10 mL measuring flask, volume is sufficient with methanol to the limit mark [12].

8. Determination of Antioxidant Activity

The solution to be tested is pipeyted as much as 1 mL of each concentration then put in a vial bottle, added with a DPPH solution of 1.5 ml shaken until homogeneous, incubated in a place protected from light for 30 minutes, then read the absorption at a wavelength of 517 nm [12].

9. Serum Physical Stability Test

This test was carried out by organoleptic physical observation, dispersibility test, irritation test, pH test, homogeneity test and preference test of the best formulation on the antioxidant serum preparation of *Centella asiatica* extract for thirty days which was tested once a week with different storage temperatures, namely at room temperature (15-30°C), hot temperature (< 40°C), and cold temperature (2-8°C) [13].

Data Analysis

The determination of antioxidant activity using the DPPH method is expressed by the damping value of DPPH (IC50), the greater the damping value, the greater the value of antioxidant activity[14]. The percentage of DPPH inhibition activity in serum samples follows the formula:

$$\%\ inhibisi = \frac{absorbansi\ kontrol - absorbansi\ sampel}{absorbansi\ kontrol}\ x\ 100\%$$

IC50 is calculated using the linear regression equation y=ax+b, sample concentration as x-axis and % inhibition as y-axis, IC50 value is calculated using the formula:

$$y=ax + b$$

$$5=ax + b$$

$$(x)\ IC50= \frac{5-b}{a}$$

The IC50 value will determine the antioxidant activity of the serum preparation produced, namely with very strong activity if the IC50 value is less than 50 ppm, strong for IC50 with a value of 50-100 ppm, said to have moderate activity if it is worth 100-150 ppm, and if the IC50 value is 151-200 ppm then it is said that the serum has weak antioxidant activity.

RESULTS AND ANALYSIS

1. Phytochemical Screening of Phenolic Compounds

Qualitative testing for phenolic compounds in Centella asiatica extract and rose oil using $FeCl_3$ reagent produced a green color change. This result confirms the presence of phenolic hydroxyl groups in both active raw materials

2. Antioxidant Activity Test (DPPH Method)

The physical stability and antioxidant activity test of the antiaging serum combination of *Centella asiatica* extract and rose oil was carried out with the aim of determining the ability of the preparation in the specifications that have been set whether the preparation is in accordance with quality standards throughout the storage period to ensure the quality of the product. In the serum preparation, a physical stability test was carried out and the antioxidant level was determined using UV-Vis spectrophotometry. Serum storage is carried out at room temperature (15-30°C), hot temperature (< 40°C) and cold temperature (2-8°C) by monitoring the serum physical properties test from week 0 to week 4. The active ingredient of the

serum is Centella asiática extract which is taken by maceration. By soaking or maceration, there will be a fairly wide and even contact to produce a more maximum withdrawal of active substances, where due to the difference in pressure between the inside and outside of the sample cell will experience a breakdown of the cell walls and membranes, as a result of which the active substance can be extracted evenly throughout the 96% ethanol as an organic solvent used for the extraction process [10]. The selection of other ingredients used in preparations in the form of rose oil, due to This oil is widely used as a skin moisturizer, and to treat wrinkles. Another benefit is to brighten the skin, tighten the skin and can also fade the hyperpigmentation that occurs on the skin due to the vitamin C content in it [3]. The active compounds of rose extracts and oils that act as the greatest antioxidants are the phenol group. From the results of the test of the identification of phenol compounds in *Centella asiatica* and rose oil using the $FeCl_3$ reagent, it produced a green color. The discoloration occurs due to a reaction between hydroxyl compounds in phenols and reagents [15]. The purpose of the compound identification test is to determine the content of the active substance, namely phenol compounds, the test is carried out qualitatively with the addition of reagents.

Centella asiatica extract obtained is then combined with rose oil in a serum preparation. Serums that are light on the skin have a high concentration of active ingredients such as antioxidants and oxfoliators. Skin problems such as dark spots, dry skin lines and fading acne scars can be treated with a serum. The serum formulation from natural ingredients such as gotu gotu extract and rose oil produces a serum that is safe to use on the skin. The next stage is to conduct a physical test of serum preparations [16] and the physical stability of the serum to determine whether the serum made meets the requirements of the physical properties test.

3. Physical Properties and Stability Test of the Spray Gel Serum

a. Organoleptic and Homogeneity Test

As a physical parameter to determine the texture or shape, color, smell or aroma of the preparation. Organoleptic parameters are carried out by looking at color, smelling, and looking at the shape/texture of the natural gel made. From the results of organoleptic observations on the preparation after 4 weeks of storage, it was shown that the serum spray gel preparation produced a yellowish-green color that appeared transparent and thick. The aroma is that it smells like rose oil. Organoleptic preparations show the same results at different temperature storage.

Table 1.
Results of Serum Organoleptic Test Room Temperature, Hot Temperature and Cold Temperature

Temperature	Organoleptis	Organoleptic Test Results Weeks 0 - 4
Room	Shape	Thick
	Color	Transparent yellowish green
	Smell	Specialty rose oil
Hot	Shape	Thick
	Color	Transparent yellowish green
	Smell	Specialty rose oil
Cold	Shape	Thick
	Color	Transparent yellowish green
	Smell	Specialty rose oil

In addition to the organoleptic test, homogeneity testing is also carried out in table 2, this test aims to see the distribution of particles from the preparation where the condition of a preparation is said to be homogeneous, namely it must not contain coarse materials that can be felt [17]. In the results of the homogeneity check of serum preparations stored for 4 weeks at different temperatures, it was found that the preparations were evenly mixed because they had well-distributed particles, homogeneous preparations did not have

coarse grains or lumps in the preparation.

Table 2.
Serum Homogeneity Test Results for Room Temperature, Hot Temperature and Cold Temperature

Week	Homogeneity Temperature Test Results		
1	Room	Hot	Cold
0	Homogeneous	Homogeneous	Homogeneous
1	Homogeneous	Homogeneous	Homogeneous
2	Homogeneous	Homogeneous	Homogeneous
3	Homogeneous	Homogeneous	Homogeneous
4	Homogeneous	Homogeneous	Homogeneous

b. pH Test

The results of the pH measurement of the preparation carried out in week 0 obtained a pH value of 5, where the pH of the preparation corresponds to the pH of the facial skin, which is 4.5-6.5. This result is fixed which means that the preparation does not experience any change in pH value after storage for 4 weeks at different temperatures. In preparations that have a pH outside the pH interval, the skin is feared to cause scaly skin or even irritation can also cause the skin to feel slippery, dry quickly, and can affect skin elasticity. From the tests that have been carried out, the preparation is still within the range of the pH value within the safe limit for topical preparations, which is around 4.5-6.5 [16].

c. Sticky Dispersion Test

This test is done with the aim of calculating how long it takes for the serum to spread and adhere to the surface of the skin. Good adhesion dispersion time is under 10 seconds [16]. The results of the adhesion dispersion test were carried out in 3 replications and were calculated at the time of dripping down or not sticking. The results of the drip speed test produced in week 0 were less than 10 seconds, which was an average of 2.3 seconds. After storing at different temperatures for 4 weeks, the results at each storage temperature of the preparation have changed slightly, but the results obtained still meet the requirements. From these results, it is known that gotu gotu extract and rose oil can be formulated into serum preparations *spray gel* and the preparation can be applied well to the skin. The results of the adhesion test after being stored at each temperature for 4 weeks :

Table 3.
Serum Adhesion Test Stability Results

Week to -	Adhesion Test Results Temperature		
	Room	Hot	Cold
0	2,3	2,3	2,3
1	2,2	2,1	2,3
2	2,1	2,1	2,2
3	2,0	2,0	2,1
4	2,0	1,9	2,1
Avg.	2,12	2,08	2,2

d. Preference Test

This test is carried out with the aim of finding out whether the serum formula made is preferred by probandus. The serum is applied to the face 2 times a day after a cotton bath for 4 weeks [6]. The assessment of the preparation is carried out by providing a questionnaire based on several characteristics possessed by the preparation, namely the aroma/smell, color, and comfort of using the serum preparation *spray gel* with the highest value parameters are like, dislike, and dislike. This test was followed by 10 respondents who were randomly selected. The results were obtained that the probandus chose likes = 5, less likes = 3 and dislikes = 2. The results showed that out of 10 probandus who tried this spray gel serum preparation, they chose to dislike and dislike based on the color of the preparation because some probandus said that the color of the preparation produced was less attractive or less bright even though it seemed transparent. As for probandus who choose to like it based on the taste of the skin, when applied to the skin, according to the probandus, there is a cold sensation that they feel also because of the fragrant smell of rose oil.

Antioxidant activity tests were carried out on preparations in weeks 0 and 4 with DPPH damping, spectrophotometric antioxidant activity checks were carried out by reacting serum samples with DPPH solution[18]. The test results in week 0 were obtained with a value of y = 1.7027x + 1.6041 with a value of r = 0.9634. The relationship is obtained from the probit value in the plot result table from % inhibition which is then made a graph between the concentration log (x) and the probit (y) until the linear equation y = ax + b is obtained. There was a decrease in the absorbance value of the DPPH given to the sample with each increase in concentration, which means that free radical capture in this case DPPH has occurred by the sample. The results of the plot to the linear regression equation obtained from the serum sample before storage obtained the IC value$_{50}$ 98.72 ppm, where the IC value$_{50}$ is a number that shows the sample concentration (ppm) which can inhibit the oxidation process by 50%. When the IC value$_{50}$ The smaller it is, the higher the antioxidant effectiveness[14].

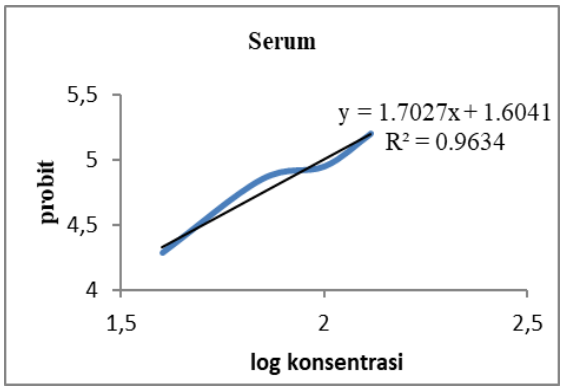
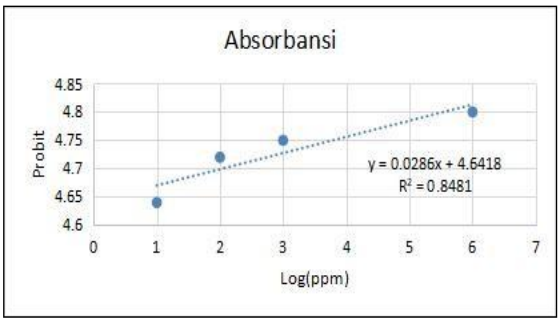


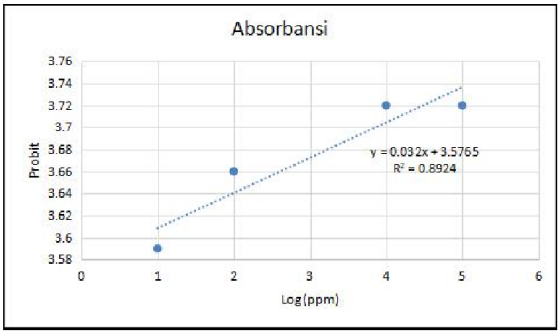
Figure 1. Serum Antioxidant Test Results

From the results obtained, it shows that the *Centella asiatica* serum and rose oil made have strong antioxidant power. This is possible because the phenol compounds found in *Centella asiatica* and rose oil work synergistically as antioxidants. This is in line with previous research that the combination of phenol compounds from two types of plants will result in a stronger IC50 value than in its single form [19]. This is because the natural antioxidants of both plants can protect the skin from the sun due to *Reactive Oxygen Species* and free radicals [11].

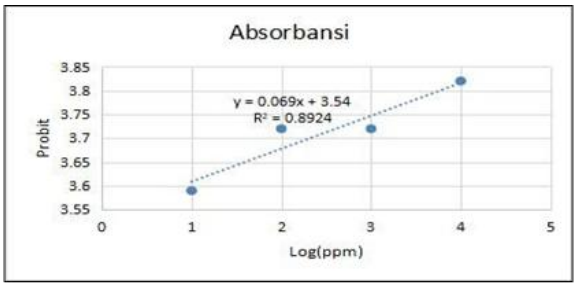
The next antioxidant activity test was carried out after the serum was stored at room temperature, cold and hot for 4 weeks. From each storage temperature, a test was then carried out in the same way as the preparation test in the 0th week of storage, and the following results were obtained:



(a)



(b)



(c)

Figure 2. Results of Linear Regression of Antioxidant Activity Test (a) Room Temperature (b) Hot Temperature (c) Cold Temperature

Table 4. Results of IC50 Serum Value After Storage

Yes	Storage Temperature	Linear Regression Equations	IC50 (ppm)
1	Room	y = 0.0286x+4.641	345,63
2	Hot	y = 0.32x+3.5765	484,41
3	Cold	y = 0.69x+3.54	271,64

Based on the results obtained, it was shown that the antioxidant activity curve of the serum preparation sample solution at room temperature (25°C) produced a value of R = 0.8481 with an IC50 value of 345.63 ppm, at hot temperature (40°C) with a value of R = 0.8924 obtained an IC50 value of 484.41ppm and at cold temperature (4°C) produced an R value = 0.8924 with an IC50 of 271.64 ppm. Results of IC values$_{50}$ The difference before and after storage shows that time and temperature affect the results of the antioxidant activity of serum preparasions. In previous research, the results showed that the antioxidant activity in centella asiatica serum had an IC50 value of 191[20]. While in this study, after adding rose oil to the formula and also conducting a stability test, the IC50 was obtained as 271.64 ppm at cold temperatures. This value indicates better antioxidant activity than serum tested at room temperature and heat.

High temperatures act as a catalyst, accelerating the breakdown of the chemical structure of antioxidant compounds. Heat breaks the double bonds in phenolic and flavonoid compounds, causing the serum to lose its free radical-fighting ability dramatically over a short period of time. In previous research on the formulation of antioxidant serum preparations of Red Dragon Fruit Ethanol Extract (Hylocereus polyrhizus) at cold temperatures, room temperature, and extreme temperatures/oven (40^0C) for a period of 4 weeks, the results showed that there was an effect of temperature and time on antioxidants. Cold temperatures have the best stability. The decrease in antioxidant activity occurs very slowly and the value remains in the strong antioxidant category until the 4th week. Oven temperature experienced the most drastic decrease in antioxidant activity. As storage time increases (from week 1 to week 4), hot temperatures damage flavonoid and polyphenol compounds in the serum. As a result, the IC50 values jumped up significantly which means the ability to ward off free radicals is drastically weakened[21].

The antioxidant effectiveness of serum preparations decreases after storage, this is evident from the greater IC50 value than before storage. Some of the factors that can cause weak antioxidant levels in serum preparations are storage time, extreme temperatures, and the presence of other compounds or additives in serum preparations so that it is possible to reduce the level of antioxidant active compounds in the preparation. Another factor that affects the decrease in the effectiveness of antioxidants after storage is environmental factors, for example light which can affect the oxidation process so that it can reduce the antioxidant activity of serum preparations. Poor treatment when making preparations that result in active ingredients and additives in preparations having more contact with the environment, is also a cause that can reduce the antioxidant activity of serum preparations that have been made [22].

DISCUSSION

The extraction of Centella asiatica herb was performed by maceration using 96% ethanol as an organic solvent. Pressure differentials between the interior and exterior of the sample cells during soaking induce cell wall and membrane breakdown, enabling optimal extraction of active phenolic constituents[23]. Alongside Centella asiatica, rose oil (Rose damascena P. Mill.) was incorporated to function as a moisturizer, brightener, and skin refresher. Rose oil contains phenolic compounds as well as non-polar components such as citronellal and geraniol, which contribute to

anti-aging benefits[24][30]. Qualitative testing with FeCl3 yielding a green coloration confirms the presence of phenolic hydroxyl groups in both ingredients[25]. The combination of phenolic compounds from Centella asiatica extract and rose oil exerts a synergistic effect in scavenging free radicals[29].

In evaluating the physical stability of the spray gel serum formulated with HPMC, chitosan, and Tween 80 bases, the preparation preserved its thick texture, transparent yellowish-green color, and homogeneity across all storage temperatures for 4 weeks. This indicates effective particle dispersion without aggregation. Maintaining a stable pH of 5 is vital as it aligns with the physiological pH range of facial skin (4.5-6.5). Formulations with pH values outside this boundary risk causing skin irritation, scaliness, or compromised skin elasticity[26][27]. Adhesion dispersion times remained below 10 seconds, which fits the standard criteria for spray gel preparations and ensures convenient topical application. Consumer preference was influenced by aesthetic color expectations and sensory application. Although the natural extract color was regarded as less vibrant by some respondents, the cooling sensation and rose fragrance served as primary positive attributes.

Antioxidant activity testing using the DPPH free radical scavenging assay indicated that the initial preparation (Week 0) possessed an IC 50 value of 98.72 ppm, placing it in the strong antioxidant category IC 50 range of 50 - 100). However, following 4 weeks of storage, a reduction in antioxidant activity was observed, marked by higher IC 50 values across all storage conditions. Storage under cold conditions (4^{o}C) provided the highest antioxidant stability with an value of IC 50 value 271.64 ppm, compared to room temperature (345.63 ppm) and hot temperature (484.41 ppm)[28].

Elevated temperatures act as catalysts that accelerate the chemical degradation of antioxidant compounds. Heat breaks double bonds in phenolic and flavonoid structures, leading to a rapid decline in free-radical scavenging capacity. In addition to temperature and storage duration, environmental factors such as light exposure, atmospheric contact during preparation, and interactions with excipients contribute to the loss of active antioxidant potency. These findings correspond with broader stability patterns in antioxidant serum formulations, where cold storage effectively minimizes the degradation rate of polyphenols and flavonoids compared to extreme thermal exposure.

CONCLUSION

Storage time and temperature affect on:

1. The physical test of the preparation is in the adhesion test, there is a change in the results even though it still meets the required range.
2. Antioxidant activity test, a decrease in antioxidant activity was observed from a greater IC value of 50 after storage.

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