ANTIOXIDANT ACTIVITY TEST ANTI AGING SERUM FROM *Centella asiatica*  EXTRACT AND ROSE OIL

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**Abstract.** *Centella asiatica* is one of the plants in the form of herbs that have been widely used as medicine. This plant contains secondary metabolites in the form of phenol. The result of other natural ingredients that contain phenol is rose oil. One of the uses of this phenolic compound is as an antioxidant, which is a compound that can counteract free radicals. Antioxidants can affect metabolism in the body, one of which is to prevent aging of the skin. The purpose of this research is to make an anti aging serum which will be tested for its physical properties and antioxidant activity. This research is an experimental research, the data collection method uses qualitative and quantitative data from laboratory experiments. The extract was made by maceration method using 96% ethanol solvent and antioxidant activity test using DPPH reduction with UV-Vis spectrophotometer instrument. From the results of the preparations made, physical tests were carried out on organoleptic, homogeneity, pH, adhesion tests and also tested for preference and irritation. The results of the physical test showed that the serum preparation met the provisional requirements for the antioxidant power test, the IC50 result was 98,72 ppm so that the serum produced had a strong antioxidant power.

*Keywords*: Centella asiatica extract, rose oil, serum, anti aging, antioxidant

1. Introduction

The use of natural ingredients, both as medicine and for other purposes, tends to increase, especially with the issue of back to nature. Traditional medicines and medicinal plants are widely used by the community, especially in preventive, promotive and rehabilitative efforts (1). One of the plants that is useful as medicine is *Centella asiatica* (2). And the secondary metabolic product of other plants that is widely used is rose oil which is obtained from the distillation of red roses.

*Centella asiatica* is one of the medicinal plants owned by Indonesia which has been used traditionally in the treatment of various diseases such as for skin diseases, stomachaches, coughs, dysentery, inflammation and antioxidants. The important and distinctive chemical constituents of *Centella asiatica* are triterpene ester glycoside compounds, namely asiaticoside and madecoside, triterpene group compounds and phenolic group compounds (2). This chemical content can be obtained in the form of extract by maceration method using 96% solvent. While rose oil contains phenolic compounds as well as other compounds such as graniol, nerol, and citronellal which can function as anti-aging(3).

The phenolic compounds contained in *Centella asiatica* and rose oil function as natural antioxidants. Antioxidants are compounds that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. As a result, cell damage can be inhibited. The antioxidant activity of phenolic compounds is mainly due to the oxidation-reduction reaction which plays an important role in absorbing and neutralizing free radicals, reducing singlet and triplet oxygen and peroxide decomposition (4). Judging from the function of the compounds it contains, namely as antioxidants, *Centella asiatica* can be used as an anti-aging. This is because natural antioxidants from plants can protect the skin from sunlight due to Reactive Oxygen Species and free radicals (4).

Antioxidants have one benefit, namely as an antidote to the symptoms of antiaging. This symptom is characterized by a decrease in skin moisture and elasticity because the skin's elasticity and ability to hold water has decreased. As a result, the face looks wrinkled, dry, rough skin and the presence of black spots, where this condition is very easily experienced by women, especially those aged 40 years and over (5). To facilitate the use of *Centella asiatica* extract and rose oil as anti-aging, it is necessary to make a preparation. The dosage form chosen was serum and physical properties were tested on the preparations made.

Antioxidant activity can be proven by testing the free radical scavenging method of DPPH (2,2-diphenyl-1-picrylhydrazyl). Although there are several methods of testing antioxidant activity, this DPPH method was chosen because it requires a small sample, is simple, easy, fast, and sensitive to evaluate the antioxidant activity of natural compounds (6).

1. Methods

Research Tools

The tools used are as follows: analytical balance, beaker glass, black jar for maceration, stirring rod, flannel, glass funnel, steam dish, test tube, measuring flask, volume pipette, blender, rotavapor, vial, measuring cup, micro pipette, cuvette, UV-Vis spectrophotometer (Ganesys 10 S).

Research Material

The materials used in this study were the *Centella asiatica* herb, aquadest, chitosan, tween 80, acetic acid, Aethanolum 96%, Methanol, DPPH, and FeCl3 5%.

Research procedure

1. Making Simplicia Powder

*Centella asiatica* herbs are collected and cleaned of dirt, then washed with running water until clean and then dried under direct sunlight. After the simplicia dried, it was mashed and sieved with a mess size of 60 to obtain a fine simplicia powder. The simplicia powder obtained was stored in a clean, dry and tightly closed container.

1. Phytochemical Screening of Phenol Compounds

The simplicia powder was weighed as much as 2 g, added with distilled water and heated for about 10 minutes. The results obtained were filtered to separate the extract and the dregs. Then as much as 2 ml of the sample in a test tube was added 5 drops of FeCl3 to produce a blue or green-black color (7).

1. Production of *Centella asiatica* Herb Ethanol Extract

150 g of *Centella asiatica* herb simplicia powder was extracted with 750 ml of 96% ethanol solvent, soaked for ± 5x24 hours while stirring occasionally, after that it was filtered to separate the dregs and the filtrate. Furthermore, the filtrate is evaporated to obtain a thick extract. Performing an ethanol-free test on the extract by inserting a small amount of extract into a test tube, then adding 2 drops of acetic acid and 2 drops of H2SO4. Observing the change in odor, if there is no smell of ethyl acetate (ester), the extract is free from ethanol. The thick extract obtained was weighed and stored in a tightly closed container (8).

1. Serum Making

The preparation was made by weighing the HPMC then developed in distilled water, stirred homogeneously to produce a gel base and then allowed to stand for a while so that the foam disappeared. Chitosan was dissolved in 0.5% acetic acid solution then mixed with the extract and rose oil that had previously been dissolved in tween 80. Mix until homogeneous.

1. Serum Preparation Test
2. Organoleptic Test

Testing by visual observation of the serum.

1. pH test

Testing pH using a pH meter or pH stick and compared with the pH literature.

1. Homogeneity test

Testing on the homogeneity of the serum.

1. Adhesion Measurement

This test is carried out by spraying the preparation on the arm at a distance of 3 cm. After that it is counted for 10 seconds to see if the preparation sticks or droplets from the spray drip down.

1. Irritation Test

The technique used in this irritation test is an open patch test on the inner forearm of 10 panelists. The open patch test was carried out by applying the preparation made at the location of the attachment with a certain area (2.5 x 2.5 cm), leaving it open and observing what happened. This test was carried out once a day for three consecutive days. A positive irritation reaction is indicated by the presence of redness, itching, or swelling on the skin of the treated forearm. The presence of red skin is marked (+), itching (++), swelling (+++), and those that do not show any reaction are marked (-).

1. Like Test

The test is to determine the respondent's level of preference for the results of serum preparations.

1. Analysis of Antioxidants in Serum Preparations
2. Preparation of blank solution

The blank solution used was methanol. Recording was carried out on the absorbance at a wavelength of 515 nm.

1. Preparation of DPPH solution

DPPH solution was prepared by dissolving DPPH with a concentration of 40 g/mL, in methanol made fresh and protected from light. A total of 10 mg of DPPH was dissolved with methanol in a 10 mL volumetric flask, shaken until homogeneous. 4 mL of this solution was pipetted and put into a 100 mL volumetric flask, then methanol was added to the limit and a reagent solution with a concentration of 40 g/mL was obtained.

1. Preparation Serum Master Solution (2000 ppm)

The serum was weighed as much as 100 mg, dissolved in methanol and then put in a 50 mL volumetric flask, the volume was filled with methanol to the limit mark.

1. Preparation Serum Series Test Solutions (50, 100, 200 and 400 ppm)

The master serum solution was pipetted 0.25 each; 0.5; 1; 2 (mL) was put into a 10 mL volumetric flask, the volume was made up with methanol to the mark.

1. Determination of Antioxidant Activity Is done By DPPH Method

1 mL of test and control solution from each concentration was pipetted and put in a vial, then 1.5 ml of DPPH solution was added, shaken until homogeneous, then incubated for 30 minutes in a place protected from light. Next, read the absorption at a wavelength of 517 nm.

1. Antioxidant Activity Data Analysis

Determination of antioxidant activity using the DPPH method is expressed by the DPPH attenuation value (IC50), the greater the attenuation value, the greater the antioxidant activity value. The percentage of DPPH inhibitory activity in serum is expressed by the formula:

The percentage of inhibition data was then graphed between the concentration (x) and % inhibition (y) in order to obtain a linear regression equation y= ax + b. By entering the value of y = 50, the IC50 value will be obtained. IC50 is the concentration required to reduce DPPH by 50%. Then IC50 was calculated using a linear regression equation, the sample concentration as the x-axis and % inhibition as the y-axis. From the equation y= a+bx, the IC50 value can be calculated using the formula:

y= ax + b

50=ax + b

(x) IC50=

1. Results and Discussion

This study aims to determine the physical properties and antioxidant power of *Centella asiatica* extract serum and rose oil.

Extraction was carried out by maceration using 96% ethanol as solvent. Maceration was carried out for 5 days with the aim that the active substance in the simplicia was completely dissolved into the solvent, the principle used in the extraction was an equilibrium reaction between the active substance and the solvent. During the immersion process, the cell walls and cell membranes will break down due to the pressure difference between the outside of the cell and the inside of the cell so that the secondary metabolites in the cell will be dissolved in the organic solvent used (9). Meanwhile, rose oil is obtained directly from the laboratory. To determine the content of the active substance, namely phenolic compounds, a qualitative test was carried out. The test results obtained a blackish green color after the simplicia was added with FeCl3 reagent, this indicates that the tested sample contains phenolic compounds (10).

The serum preparations that have been made are then evaluated for preparations to determine whether the serum made is in accordance with the standard when viewed from the physical tests and irritation tests that have been carried out. The results of the physical tests carried out are as follows:

**Table 1. Table of physical test results for serum preparations**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No | Test Criteria | Test Results | | |
| Replication 1 | Replication 2 | Replication 3 |
| 1 | Organoleptic test | | | |
| * Shape | Thick | | |
| * Color | Greenish Yellow | | |
| * Smell | Rose oil smell | | |
| 2 | Homogeneity test | Homogeneous | | |
| 3 | pH test | 5 | 5 | 5 |
| 4 | Adhesive test | 2 second | 3 second | 2 second |
| 5 | Irritation test | No irritation | | |

1. Organoleptic Test

Organoleptic observations of the preparations showed that the gel preparations produced were greenish yellow in color and viscous. The aroma that is generated is the distinctive smell of rose oil.

1. Homogeneity Test

The results of the homogeneity of the spray gel preparations that have been made are homogeneous because there are no coarse grains, this can be seen from the visual test carried out by dripping a small amount of the gel preparation onto the surface of the glass slide, then covering it with another glass preparation.

1. pH test

The pH test aims to evaluate the pH of serum preparations so that they are suitable. The pH test was carried out 3 times as shown in table 1. The pH value of the preparation has met the requirements, which is in the range of 4.5-6.5 (4), thus the serum preparation made is safe to be applied to the skin.

1. Adhesion Test

The serum adhesiveness test aims to calculate how long it takes the serum to spread and adhere to the skin surface. The results of the adhesive dispersion test were obtained in 3 replications at 10 seconds dripping down or not sticking. The resulting drip speed result is less than 10 seconds (4).

1. Irritation Test

Irritation test is carried out on serum preparations made with the intention of knowing whether the preparations can cause irritation to the skin or not. Irritation can be divided into two categories, namely primary irritation, which will appear immediately after the attachment or contact with the skin, and secondary irritation, which occurs only a few hours after touching or sticking to the skin.

Based on the results of the irritation test on 10 respondents by applying the preparation made on the forearm to all respondents, all respondents gave negative results or there were no signs indicating an irritation or allergic reaction such as redness, itching and swelling of the preparations made.

1. Like Test

This test was carried out with the aim of knowing whether the serum formula made was preferred by the respondents. Assessment of the preparation was carried out by giving a questionnaire based on several characteristics possessed by the preparation, namely aroma/smell, color, and convenience of using spray gel preparations with the highest value parameters being likes, dislikes, and dislikes. This test was followed by 10 respondents who were chosen randomly. The results showed that respondents chose like = 5, disliked = 3 and did not like = 2.

The results of the preference test shows that of the 10 respondents who tried this serum gel preparation, they chose less like and dislike based on the color of the gel preparation. because the panelists said that the color of the preparation produced was less attractive. Meanwhile, for respondents who chose to like it based on the taste on the skin and the fragrant smell of rose oil produced, according to the respondents they felt a cold sensation when applied to the skin.

Antioxidant Activity Test With DPPH Method

After the physical test of the preparation was carried out, the antioxidant activity test was carried out with DPPH. Spectrophotometric examination of antioxidant activity was carried out by reacting serum samples with DPPH solution. The absorbance measurements of the samples were carried out at concentrations of 50 ppm, 100 ppm, 200 ppm and 400 ppm. The absorbance measurement data at 517 nm is shown in the following figure:

**Table 2. Antioxidant test results**

|  |  |  |
| --- | --- | --- |
| Concentration | Concentration log | Probit |
| 50 | 1,69 | 4,42 |
| 100 | 2,00 | 4,67 |
| 200 | 2,30 | 5,03 |
| 400 | 2,60 | 5,47 |

**Figure 1. Serum linear regression results**

From the picture above, the value of y = 1,7027x + 1,6041 is obtained with a value of r = 0,9634. The relationship was obtained from the % inhibition and the concentration used. In the absorbance results there is a decrease in the absorbance value of DPPH which is sampled at each increase in concentration. The decrease in the absorbance value of DPPH means that there has been DPPH radical capture by the sample. By capturing these radicals, the double bonds in DPPH are reduced, causing a decrease in absorbance.

Antioxidant activity was measured as a decrease in the absorption of the DPPH solution due to the addition of the sample. The absorption value of the DPPH solution to the sample was calculated as percent inhibition (% inhibition). The results are plotted into the obtained linear regression equation so that the IC50 value is 98,72 μg/mL..

The smaller the IC50 value, the higher the antioxidant activity. Specifically, a compound is said to be a very strong antioxidant if the IC50 value is less than 50 ppm, strong for IC50 is 50-100 ppm, moderate if it is 100-150 ppm, and weak if the IC50 value is 151-200 ppm (11). From the results, it was found that the serum made had strong antioxidant power.

From the results, it was found that the serum made has strong antioxidant power. The phenolic compounds contained in the *Centella asiatica* extract and rose oil can function as natural antioxidants, so that they can be used as anti-aging in cosmetic preparations, especially for facial skin needs.

1. **Conclusion**

From the results obtained, the serum *Centella asiatica* extract and rose oil has met the requirements of the physical test and has a strong antioxidant power.

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