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FORMULASI SEDIAAN SERUM ANTIOKSIDAN EKSTRAK ETANOL DAUN ALPUKAT (*Persea americana* M.)

FORMULATION OF ANTIOXIDANT SERUM PREPARATION OF AVOCADO LEAF ETHANOL EXTRACT (*Persea americana* M.)

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ABSTRAK

Daun alpukat berkhasiat sebagai antioksidan yang bekerja sebagai pendonor elektron dan bereaksi dengan radikal bebas menjadi senyawa yang stabil. Penelitian ini bertujuan untuk mengetahui kualitas fisik, stabilitas sediaan serum ekstrak daun alpukat dengan variasi karbopol, dan untuk mengetahui apakah sediaan serum ekstrak daun alpukat memiliki aktivitas antioksidan. Penelitian ini menggunakan 5 formulasi, yaitu kontrol negatif, kontrol positif, ekstrak etanol daun alpukat konsentrasi 10% dengan variasi konsentrasi karbopol 0,5%, 1%, dan 1,5%. Metode yang digunakan adalah metode DPPH dengan menggunakan spektrofotometri UV-Vis. Evaluasi sifat fisik sediaan terdiri dari uji organoleptis, homogenitas, viskositas, pH, daya sebar, dan stabilitas. Hasil penelitian menunjukkan bahwa sediaan serum dengan variasi konsentrasi dapat memberikan pengaruh terhadap nilai kualitas fisik dan stabilitas, serta sediaan serum ekstrak daun alpukat memiliki aktivitas antioksidan yang tergolong antioksidan kuat dengan nilai IC50 pada formula 2 sebesar 89,256 ppm, formula 3 sebesar 87,868 ppm, dan formula 4 sebesar 89,256 ppm.

Keywords:

Avocado leaf extract
Antioxidant
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ABSTRACT

Avocado leaves are efficacious as antioxidants that work as electron donors and react with free radicals into stable compounds. This study aims to determine the physical quality, stability of avocado leaf extract serum preparations with carbopol variations, and to determine whether avocado leaf extract serum preparations have antioxidant activity. This study was used 5 formulations, namely negative control, positive control, avocado leaf ethanol extract concentration of 10% with carbopol concentration variations of 0.5%, 1%, and 1.5%. The method was used the DPPH method using UV-Vis spectrophotometry. Evaluation of the physical properties of the preparation consisted of organoleptic, homogeneity, viscosity, pH, spreadability, and stability tests. The results had shown that serum preparations with variations in concentration can have an affect on the value of physical quality and stability, and avocado leaf extract serum preparations had antioxidant activity classified as strong antioxidants with IC_{50} values in formula 2 of 89.256 ppm, formula 3 of 87.868 ppm, and formula 4 of 89.256 ppm.

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1. INTRODUCTION

The utilization of traditional plants as plants that have functional value for health has been widely carried out and the content of compounds such as phenolic compounds, flavonoids, terpenoids in plants is efficacious as antioxidants, antimicrobials, anti-inflammatory and others. Antioxidants are compounds that can stabilize free radical reactions by inhibiting chain reactions and complementing the lack of electrons possessed by free radicals. Antioxidants play an important role in maintaining immunity, and preventing continuous oxidation processes in the body [1]–[3].

One of the plants that has antioxidant activity is avocado leaves. In phytochemical research, avocado leaves contain alkaloids, flavonoids, quinones, and tannins [4]. The highest compound in avocado leaves is flavonoids. Flavonoid compounds in avocado leaves have strong abilities as electron donors, can react with free radicals to be converted into more stable compounds, end radical chain reactions and as chemopreventive agents [5], [6].

The results of the study by Rahmah *et al*., (2023) stated that the antioxidant activity of avocado leaf ethanol extract had a value of 9.24 ppm which is categorized as a very strong antioxidant [7]. From the results of this study, it can be said that avocado leaf ethanol extract can be made into a finished preparation with very strong antioxidant activity because it is less than 50 ppm.

Avocado leaf extract can be formulated additional source of antioxidants can be formulated into cosmetics to make them look more attractive. Therefore, a serum preparation containing avocado leaf ethanol extract as an antioxidant was prepared. The advantages of the serum preparation are that it has a high concentration of active ingredients so that the serum preparation is absorbed faster by the skin, provides a more comfortable effect, and is easier to spread on the skin surface because the viscosity it has is low [8], [9].

Determination of the Inhibition Concentration 50% (IC_{50}) value is a parameter for determining the antioxidant activity of a compound using the DPPH method. The DPPH method is widely used to determine the antiradical ability of a compound because the results are proven to be accurate, require few samples, are simple, fast, reliable, and practical. The IC_{50} value can be determined by measuring the absorbance using UV-Vis spectrophotometry, and after the absorbance is obtained, it is further calculated using the linear regression equation formula [10].

Carbopol is one of the additional ingredients in serum gel formulation that functions as a thickener or gelling agent [11]. The selection of carbopol as a gelling agent in serum preparations is because it is compatible with other ingredients, provides good flow properties at low concentrations, and has good stability. Therefore, this study was conducted to observe the effect of variations in carbopol concentration of 0.5%, 1%, 1.5% on the physical properties of the preparation and antioxidant activity of avocado leaf ethanol extract serum. Research on avocado leaf serum preparation as an antioxidant has great benefits for skin health, natural resource utilization, and the development of the botanical-based cosmetics industry. Further formulation, stability and efficacy studies are needed to ensure optimal benefit.

2. METHODS

2.1 Tools and Materials

The tools used in this study were an oven, maceration bottle, analytical balance, rotary vacuum evaporator, 40 mesh sieve, flannel cloth, UV-Vis spectrophotometry, a set of spreadability testers, pH meter, stirring rod, aluminum foil, mortar, stamper, beaker, measuring flask, volume pipette, water bath, moisture balance, Brookfield dV2t viscometer, vial, and stopwatch. The materials used in this study were avocado leaves, 70% ethanol, carbopol, propylene glycol, methyl paraben, triethanolamine, distilled water, DPPH, phytochemical identification reagents

2.2 Methodology

Plant determination, avocado plants were identified in the laboratory of Setia Budi University, Surakarta.

Preparation of simplicial, dried avocado leaves were made into powder using a blender and then sieved using a 40 mesh sieve.

Determination of drying loss, avocado leaf powder was weighed as much as 2 grams, then put into a moisture balance device at a temperature of 105^0C.

Preparation of extract, a total of 1000 grams of powder was placed in a dark glass bottle macerated using 10 parts of 70% ethanol. The maceration bottle was left for 3 days at room temperature and shaken every 8 hours. The maceration results were filtered with flannel and filter paper. The bottle was rinsed with 2.5 parts of 70% ethanol to wash the extract in the bottle. The filtrate obtained was concentrated with a rotary vacuum evaporator until a thick extract was obtained. The extract obtained was calculated for its yield.

Determination of extract moisture, determination of extract moisture content using the gravimetric method. The moisture content of avocado leaf extract that meets the requirements is not more than 14% [11].

Identification of the chemical content of extract

Alkaloid test, the extract was dissolved in ethanol and then divided into three tubes. One tube was given no reagent and the other two tubes were given a few drops of 2 N sulfuric acid and then tested with *Dragendorff's* reagent and *Mayer's* reagent, 4-5 drops each. The test result is positive if a red-orange precipitate forms with *Dragendorff's* reagent and a yellowish-white precipitate forms *Mayer's* reagent [12]. Flavonoid test, heat 5 mL of extract is heated in a water bath, add 0.1 grams of magnesium powder, 2 mL of alcohol solution: hydrochloric acid (1:10) and amyl alcohol solvent. Shake vigorously and allow to separate. Positive results are indicated by the presence of an orange-red color in the amyl alcohol layer [12]. Tannin test, the extract is added with methanol until completely submerged, then 2-3 drops of 1% $FeCl_3$ solution are added. Positive results are indicated by the formation of a blackish blue or blackish green color [13]. Saponin test, dissolve the extract with in hot water, shake vigorously, positive saponin is indicated by the presence of stable foam of 1-10 cm. The solution is allowed to stand 2 minutes, 2N HCl is added, if saponin is present, a stable foam is formed for about 10 minutes [14]. Steroid or triterpenoid test, the extract is added with 10 drops of glacial CH_3COOH and 2 drops of H_2SO_4. The solution is shaken slowly and left for several minutes. Steroids give a blue or green color, while triterpenoids give a red or purple color [15].

Preparation of serum, carbopol is sprinkled on 20 mL of distilled water in a mortar. The sprinkled carbopol is added with TEA, stirred until a gel mass is formed. Then dissolve

methyl paraben in propylene glycol, stirred until homogeneous. The gel base that has been formed is added with methyl paraben and propylene glycol solutions, stirred until homogeneous. After that, add the avocado leaf ethanol extract and the remaining distilled water, then stir again until homogeneous. The design of the antioxidant serum formula for avocado leaf ethanol extract can be seen in the following table:

Table 1. Design of antioxidant serum formula from avocado leaf ethanol extract

Materials	Function	Concentration %				
		F1	F2	F3	F4	F5
Extract	Active substance	-	10	10	10	-
Vitamin E	Active substance	-	-	-	-	0,1
Carbopol	Gelling agent	1	0,5	1	1,5	1
Propylene glycol	Humectant	10	10	10	10	10
Methyl paraben	Preservative	0,3	0,3	0,3	0,3	0,3
Triethanolamine	Alkali agent	2	2	2	2	2
Aquadest	Solvent	ad 100	ad 100	ad 100	ad 100	ad 100

Serum physical properties testing

Organoleptic examination, conducted to see the physical appearance of the preparation by observing the color, odor, and texture of the serum preparation. Homogeneous examination, conducted by applying a serum sample to a piece of glass or other suitable transparent material. Viscosity measurement, place 100 mL of the preparation in a beaker, then select a spindle with a specific number. Dip the spindle into the preparation until it is submerged. The spindle is set at a speed of 50 rpm [16]. pH measurement, the preparation is put into a beaker, the pH meter is put into the preparation, and the results on the pH meter screen are recorded [17]. Spreadability test, the serum is weighed 0.5 grams and then placed on the spreading power tester. Testing is carried out by calculating the spreading power when adding a load of 50 grams, 100 grams, and 500 grams [18]. Stability test, the stability of the preparation was evaluated using the cycle test method by storing the preparation at a temperature of (4° ± 2°C) and then placing it in an oven at a temperature of (40° ± 2°C) for 24 hours (1 cycle). The test was perfomed in 6 cycles [17].

Serum antioxidant activity test.

Preparation of 0.4 mM DPPH stock solution, DPPH powder was weighed as much as 15.8 mg and dissolved in ethanol *p.a* up to 100 mL in a volumetric flask. The measuring flask was covered with aluminum foil and protected from light [19]. Preparation of avocado leaf ethanol extract stock solution, the thick extract was weighed as much as 5 mg and dissolved with ethanol *p.a* up to the 100 mL measuring flask boundary mark to obtain a concentration of 100 ppm. The solution was then prepared in 5 series of dilutions of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm [16]. Preparation of avocado leaf ethanol extract serum stock solution, each serum formula was weighed as much as 1 mg and then dissolved with ethanol *p.a* up to the 10 mL measuring flask boundary mark to obtain a concentration of 100 ppm. The solution was then prepared in a series of dilutions of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm [16]. Determination of the maximum wavelength, DPPH stock solution was taken 2 mL, put into a tube and then 2 mL of ethanol

p.a. was added. The absorbance was then measured at a wavelength of 450-550 nm [15]. Determination of the operating time (OT), DPPH solution was pipetted in 2 mL, then 2 mL of ethanol *p.a*. was added. OT was determined at the maximum wavelength at 5 minute intervals until a stable absorbance was obtained [19]. Antioxidant activity test, the stock solution (ethanol extract of avocado leaves and serum ethanol extract of avocado leaves) was prepared in 5 series of dilutions of 2 mL each, 2 mL of DPPH solution was added to a 5 mL volumetric flask, and ethanol was added to the limit mark. The mixture was incubated for the operating time obtained previously. Then read the absorbance at the maximum wavelength obtained [18]. Determination of IC_{50}, the determination of IC_{50} was perfomed from the results of absorbance measurements at 5 concentrations series to obtain percentage inhibition. The results of the percentage inhibition calculation are then used to find the linear equation and IC_{50} value.

3. RESULTS AND DISCUSSION

The calculated results of the extract yield was 12.7% and positively contained alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids. Flavonoid glycosides are one of the glycosides that act as antioxidants. The drying loss percentage obtained was 8.03%, while the extract water content was 9.102%. The results of the drying loss percentage and water content met the requirements because the drying loss result was not more than 10% and the extract water content was not more than 14% [11]. The purpose of the drying loss and water content test was to determine the limit of water content contained in the avocado leaf powder and extract.

The serum preparation that had been made was tested for physical quality and stability. Physical quality and stability testing is part of the development and production of a preparation to ensure that the preparation meets all criteria for safety, effectiveness, and consistent quality. Testing the physical quality of serum preparations includes organoleptic examination, homogeneity, viscosity, pH, and spreadability. The difference in the consistency of each formula preparation is influenced by the concentration of carbopol as a gelling agent, where the higher the concentration used, the thicker the preparation will be. The results of the organoleptic test of the serum preparation are shown in table 2.

Table 2. Results of the physical quality testing of the serum formulations.

Formula	Organoleptic			Spreadability Test (cm)			pH Test	Viscosity Test (cPs)
	Consistency	Color	Odor	Load 0	Load 50 g	Load 100 g		
1	Slightly thick	Transparent	Odorless	5.2	5.6	6.6	5.51	750
2	Liquid	Dark brown	Odor of extract	7.3	8	8.8	5.96	500
3	Slightly thick	Dark brown	Odor of extract	5.1	5.8	6.8	5.23	700
4	Thick	Dark brown	Odor of extract	5	5.4	6	4.45	900
5	Slightly thick	Clear cloudy	Odorless	5.1	5.5	6.1	5.23	800

Descriptions:
Formula 1 : serum without avocado as a negative control.
Formula 2 : 10% avocado leaf extract serum with 0.5% carbopol.

Formula 3 : 10% avocado leaf extract serum with 1% carbopol.
Formula 4 10% avocado leaf extract serum with 1.5% carbopol.
Formula 5 : vitamin E serum as a positive control.

The results of the serum homogeneity test of all formulations 1-5 were homogeneous because there were no coarse grains visible in the transparent, and the correct manufacturing process so that all formulations were evenly mixed. The purpose of the homogeneity test was to determine the uniformity of the distribution of materials in the formulation because the homogeneity of the active substance in the formulation can affect the effectiveness of the preparation.

The spreadability test aims to determine the spreadability of the preparation when applied to the skin, easy to clean, and easy to absorb by the skin. Based on the spreadability results, it can be seen that formula 2 with 0.5% carbopol has a spreadability value of more than 7 cm which does not meet the requirements. While formulas 1, 3, 4, 5 have spreadability values that meet the requirements where the results are 5-7 cm. The spreadability value can be influenced by the viscosity of the preparation, the thicker the preparation, the higher the viscosity and the lower the spreadability value. The results of the spreadability test are shown in Table 2.

Based on the pH measurement results, formulas I-V have serum preparation pH values ranging from 5.96 to 4.45. The pH test was performed to ensure the safety of the preparation on the skin, since a pH that is not compatible with the skin's pH can cause irritation [20]. It can be observed that the pH values of formulas 2 to 4 have decreased. The pH test results for the serum preparation are shown in Table 2.

Based on the viscosity measurement results, it can be observed that the lower the concentration of carbopol, the lower the resulting viscosity. This is because carbopol functions as a gelling agent that can increase viscosity and stabilize topical formulations [21]. The required viscosity for a good serum formulation is 230–1150 cPs, as shown in Table 2, where all five formulations meet the requirements. The differences in serum formulation viscosity for each formula can be observed in Table 2.

The formulations stability test is designed to evaluate the physical stability of a product over six cycles. Based on the organoleptic examination, the formulation did not show any changes. The results of the stability testing for spreadability revealed that all formulas experienced an increase in spreadability values. Formula 2, with a carbopol concentration of 0.5%, did not meet the requirements as its spreadability exceeded 7 cm. The acceptable spreadability range for a good formulation is 5–7 cm.

Based on the results of the pH and viscosity tests, it can be seen that after the stability test is carried out, the preparation has a decrease in pH and viscosity values. Formula 4 has a pH value that does not meet the requirements, which is less than 4.5, because the requirements for a good pH value for serum preparations are 4.5-6.5. One of the reasons is because carbopol as a thickener is acidic, and the concentration of carbopol used is high. The stability test of the preparation is tested by the cycle test method by using extreme storage temperatures, namely 4°C and 40°C. Storage at extreme temperatures may cause changes in the physical and chemical properties of the preparation.

The antioxidant activity test was performed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method with UV-Vis spectrophotometry, maximum wavelength 517 nm. The antioxidant activity can be measured from the IC_{50} (Inhibition Concentration) value. The lower the IC_{50} value, the higher the antioxidant activity. IC_{50} value is a parameter that

indicates the concentration of the extract can inhibit the activity of free radicals about 50%.

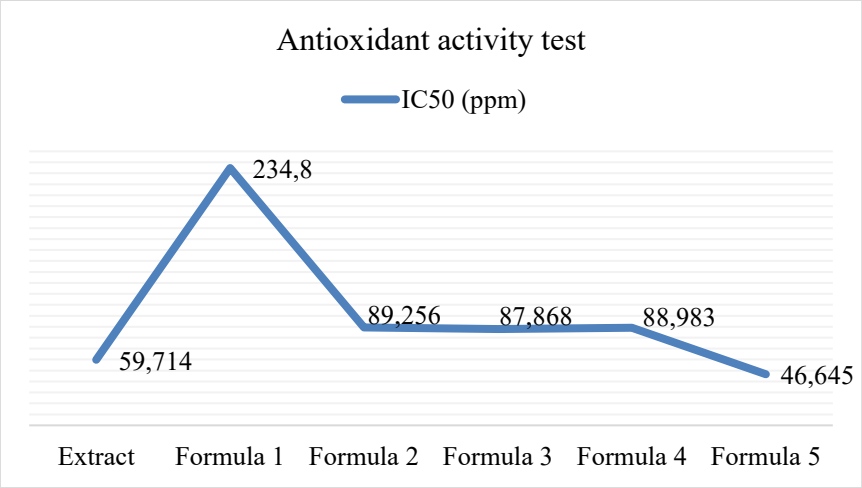

Figure 1. Graph antioxidant activity test results of avocado leaf ethanol extract.

Descriptions:
Formula 1 : serum without avocado as a negative control.
Formula 2 : 10% avocado leaf extract serum with 0.5% carbopol.
Formula 3 : 10% avocado leaf extract serum with 1% carbopol.
Formula 4 10% avocado leaf extract serum with 1.5% carbopol.
Formula 5 : vitamin E serum as a positive control.

Based on the IC_{50} value shown in the figure 1, it can be seen that avocado extract, formula 2, formula 3, and formula 4 are classified as strong antioxidants because the IC_{50} value is in the range of 50-100 ppm [22]. Formula 1 as a negative control is declared to have no antioxidant activity because the weakest antioxidant activity is 151-200 ppm. While formula 5 as a positive control containing vitamin E is classified as a very strong antioxidant because the antioxidant activity value is <50 ppm. The results of the antioxidant activity test of serum preparations were analyzed using the *Krusskall Wallis* test SPSS and obtained a sig value of 0.061 greater than 0.05, which means that there is no significant difference between the formulas of carbopol concentration variation (formulas 2-4), so it can be concluded that the carbopol concentration variation does not affect the antioxidant activity of serum preparations of avocado leaf ethanol extract. Based on the data analysis and conclusions, the author suggests that further research should be conducted to test the antioxidant activity using other methods in vivo such as TBARS (Thiobarbituric Acid-Reactive Substance), to test the antioxidant activity during the storage period to determine whether the length of storage affects the antioxidant activity of the preparation, and to conduct research by preparing serum preparations from fractionated avocado leaf extract (*Persea americana* M.).

4. CONCLUSION

Based on the results obtained, it can be concluded that variations in carbopol concentration can affect the physical quality and stability of the preparation, and serum preparations of avocado leaf ethanol extract with a concentration of 10% have strong

antioxidant activity with an IC_{50} value of formula 2 of 89.256 ppm, formula 3 of 87.868 ppm, formula 4 of 88.983 ppm.

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