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Research Article

The Antioxidant Activity of Avocado (*Persea americana* Mill) Seed Extract and Lotion Preparation

Mindiya Fatmi*, Asri Wulandari, Bina Lohita Sari, Honifa, Andrea Palleva Widjaya

Department of Pharmacy, Universitas Pakuan, Bogor, Indonesia, 16143

mindiya.fatmi@unpak.ac.id

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ABSTRACT

Excessive exposure to free radicals can cause skin to look dry and thin. Avocado seeds are a part of the fruit that is very rarely used and is even considered waste by society. Avocado seeds are known to contain good antioxidant activity to ward off free radicals. Topical preparations that are widely used to protect the skin from exposure to free radicals, one of which is lotion preparations with natural ingredients that contain antioxidants. This research aims to determine the antioxidant activity of avocado seed extract with different concentrations, formulate avocado seed extract lotion, distribute avocado seed extract lotion preparations, and determine the antioxidant activity of avocado seed extract lotion. The method used in this research is the in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Each avocado seed was extracted with ethanol 50 %; 70 %; and 96 %. The best antioxidant results were made into cream preparations in 3 formulas, namely F1 0.01 %; F2 0.05 %; and F3 0.1 %. The best antioxidant test results were obtained from avocado seed extract with ethanol 70 % and an IC_{50} of 20.68 ppm with very strong antioxidant intensity. The three lotion formulations met the evaluation requirements for topical preparations and provided an antioxidant value of F1 of 128 ppm, F2 of 72 ppm, and F3 of 55 ppm. The conclusion of this research was that the highest antioxidants in avocado seed extract were found in the 70 % ethanol extract and in the formula 3 lotion preparation.

Keywords: Avocado seed extract; Antioxidant; DPPH; Ethanolic extract; Lotion

INTRODUCTION

Skin is the body's main barrier for protection against external influences, one of which is free radicals. Exogenous free radicals can come from pollution, UV and X-rays, pesticides, and cigarette smoke, while endogenous free radicals come from within the body itself, such as autooxidation, enzymatic oxidation, and respiratory bursts (Irianti et al., 2021). Free radicals are atoms or molecules that contain one or more unpaired electrons in their outer orbit. These molecules include hydrogen atoms, transition metals, and oxygen molecules. One or more unpaired electrons in this molecule easily attract magnetic fields, making it very reactive and starting a chain reaction that causes continuous damage (Yuslianti, 2018).

Excessive exposure to free radicals can cause the skin to become dry and thin, resulting in the appearance of fine lines or wrinkles, the appearance of skin pigmentation, reduced firmness, and dullness (Sari, 2015). High exposure to free radicals from the environment means additional antioxidants are needed from outside the body to maintain body health. Sources of antioxidants can come from fruit and vegetable intakes of beta-carotene, vitamin C, vitamin E, folic acid, flavonoid compounds, and antioxidant compounds from the phenolic group (Ilmiah et al., 2023).

Antioxidants function by contributing one electron to an oxidant compound, thereby inhibiting its activity. The body needs antioxidants to defend itself

against free radical attacks. Antioxidants are chemical compounds or components that, in certain levels or quantities, are able to inhibit or slow down damage caused by the oxidation process (Sayuti & Yenrina, 2015). The way it works is to stop free radical reactions from metabolism in the body or from the environment (Meigaria et al., 2016).

Continuous exposure to free radicals can have a negative impact on skin health. These radical compounds can damage collagen fibers and the dermis matrix of the skin, causing the skin to become dry, wrinkled, and scaly, and they can even cause premature aging (Purwaningsih et al., 2014). To prevent the negative impact of free radicals, it is necessary to design a practical and comfortable cosmetic preparation for skin care and protection, for example, lotion. Lotion preparation has low consistency, is quickly absorbed into the skin surface, is easy to spread, easy to rinse with water, and dries quickly, so it does not interfere with activities. In addition, lotion preparations can be formulated using active substances from natural ingredients (Karim, 2022). Compared to cream, lotion is easier to make because its physical properties are thinner and the heating and cooling time is shorter (Oktaviasari & Zulkarnain, 2017).

The avocado plant is widely known for its delicious taste and is loved by all ages. Based on research results, Suhaenah (2019) stated that avocado seed extract has a concentration of 1000 ppm with an SPF value of 8.02, which is included in the maximum protection category. The 70 % ethanol extract of avocado seeds has an IC_{50} value of 41.5 ppm, which is classified as a strong antioxidant (Sutrisna et al., 2015). Avocado seeds contain secondary metabolite compounds, namely alkaloids, saponins, tannins, flavonoids, phenols, and steroids, which have benefits as antioxidants (Azzahra, 2022).

In this study, ethanol solvent was used with concentrations of 50 %, 70 %, and 96 %. Differences in polarity can affect the antioxidant activity of the extract. Using organic solvents at high concentrations during extraction may not necessarily increase antioxidant activity (Novianti, 2016). From the best IC_{50} value, lotion was then made with three concentration variations.

METHODS

Equipments

The tools used in this research include brown bottles, analytical scales (*And*®), blender, pH meter (*Ohaus*®), oven (*Memmert*®), UV-Vis spectrophotometer (*Thermoscientific*®), blender, filtrate filter, homogenizer, volumetric flask (*Pyrex*®), 60 mesh sieve (PT Pharmeq), and glassware*(pyrex*®).

Materials

The materials used in this research include: avocado seed extract, stearic acid (Brataco chemical), liquid paraffin (Brataco chemical), *triethanolamine* (Lug chemical), glycerin (Brataco chemical), cetyl alcohol (Merck), methyl paraben (Brataco chemical), *rose oil*, aquadest, BHT (*butylated hydroxytoluene)* (samiraschem), ethanol (Brataco), methanol pro analyst (Merck), phytochemical screening reagent.

Avocado Seed Extraction

Avocado seed powder was extracted using the maceration method with varying ethanol solvent concentrations of 50, 70, and 96 % and a ratio of 1:10 w/v. A total of 200 g of avocado seed powder was weighed and then was put into a macerator container. Then it was added 1000 mL of solvent and shaken for 10 minutes. Then let it sit for the next 24 hours, then filter using filter paper, separating the filtrate from the residue.1000 mL of solvent, shaken for 10 minutes then left for 24 minutes. The macerate was filtered using filter paper and separated between the filtrate and the residue. After 24 hours, the preparation was filtered, and the residue was then remaceration twice. The filtrate obtained was collected and precipitated, then filtered again. The extract obtained is then placed in a rotary evaporator and dried in a vacuum to produce a dry extract. Dry avocado seed extracts furthermore used to make lotion formulas and other tests include a standardization process, phytochemical screening tests, water content tests, and ash content tests (Kementrian Kesehatan RI, 2020; Hanani, 2015).

Preparation of Avocado Seed Extract Lotion

The way to make lotion uses a heating method that is divided into an oil phase and a water phase, each of which is heated at a temperature of 40-70 °C. The oil phase consists of stearic acid, liquid paraffin, and cetyl alcohol, which were heated in a beaker. The water phase consists of triethanolamine and a glass of hot water in it. In a separate container, methyl paraben was dissolved in propylene glycol. Water phase and the oil phase was mixed then homogenize. The mixture of methyl paraben and propylene glycol then added to

make the basic lotion and the different concentration of avocado seed extract was added to the basic lotion, *rose oil* was added as a additive fragrance. Finally, distilled water was added until it reaches a total volume of 100 mL. The avocado seed extract lotion preparation was evaluated using organoleptic test parameters (physical homogeneity, pH, spreadability, and stickiness). Then the antioxidant activity of the seed extract lotion preparation was carried out. The avocado seed extract lotion formula can be seen at Table 1.

Avocado Seed Extract Antioxidant Activity Test

The 100 ppm test stock solution was made into a standard series with concentrations of 10, 20, 30, 40, and 50 ppm. At this concentration, 1, 2, 3, 4, and 5 mL of the solution were pipetted, and each was put into a 10 mL flask that had been lined with aluminium foil, and then 1 mL of 100 ppm DPPH solution was added. The test solution was left for 40 minutes in a dark room at room temperature (25-30 ºC). Then the absorption was measured at a wavelength of 515.5 nm using an Ultraviolet-Visible Spectrophotometer (UV-Vis).

BHT Antioxidant Assay

BHT is a synthetic antioxidant used as a comparison in this research. The 100 ppm BHT main solution was made into a standard series with concentrations of 1, 2, 3, 4, and 5 ppm. Each was put into a 10 mL flask that had been coated with aluminium foil, and then 1 mL of 100 ppm DPPH solution was added. The test solution was left for 40 minutes in a dark room at room temperature (25–30 ℃). Then the absorption was measured at a wavelength of 515.5 nm using an ultraviolet-visible (UV-Vis) spectrophotometer.

Antioxidant Activity Test of Avocado Seed Extract Lotion

The 100 ppm lotion stock solution was made into a standard series with a concentration of 10, 20, 30, 40, and 50 ppm. At this concentration, 1, 2, 3, 4 and 5 mL of solution were pipetted. Put each into a 10 mL flask that has been coated *aluminium foil* then added 1 mL of 100 ppm DPPH solution. The test solution was left for 40 minutes in a dark room at a room temperature (25-30 ºC). Then the absorption was measured at a wavelength of 515.5 nm using an Ultraviolet-Visible (UV-Vis) spectrophotometer.

IC_{50} Antioxidant Activity Test

The antioxidant activity value can be obtained by calculating the immersion value of the DPPH compound using the following formula (1). Sample concentration and % antioxidant activity were plotted on each x and y axis to obtain a linear regression. This equation is used to determine the IC_{50} (Inhibition Concetration) value. Mark IC_{50} shows the extract concentration that can reduce 50 % absorption intensity so that it can reduce oxidation activity at 50 % (Sanjaya et al., 2020).

$$\% \text{ Aantioxidant activity} = \frac{Ab - As}{Ab} \; x \; 100\% \quad \text{..... (1)}$$

Ab = Blank Absorbance

As = Sample Absorbance

Tabel 1. Formulation of Avocado Seed Extract Lotion

Ingredient	Concentration (%)			Function
	F1	F2	F3	
Avocado seed extract	0.01	0.05	0.1	Natural active ingredient
Stearic acid	4	4	4	Emulsifier
Paraffin liquid	7	7	7	Emoliens
TEA	1	1	1	pH adjuster
Glycerin	5	5	5	Humektan
Cetyl alcohol	0.5	0.5	0.5	Emulsifier
Methylparaben	0.1	0.1	0.1	Preservative
Oleum rosae	Qs	Qs	Qs	Essence
Aquadest ad	100	100	100	Carrier fluid

Evaluation of Lotion Preparations

Evaluation of avocado seed extract lotion preparations includes organoleptic tests, including observations of the odor, colour, shape, or texture of lotion preparations containing avocado seed ethanol extract. The homogeneity test is carried out in order to determine the level of homogeneity of a lotion. The homogeneity test was carried out by placing the sample between two glass plates (Mardikasari et al., 2017). The pH test is carried out using a pH meter. To determine whether the acidity level in a preparation meets the pH requirements or not (Megantara et al., 2017). The spread ability test of the lotion is categorized as good if it ranges between 5-7 cm (Dominica & Handayani, 2019). The viscosity value of the lotion was tested using a beaker glass, and the sample was inserted up to the limit mark, then measured using spindle no. 6 at 30 rpm (Noer & Sundari, 2016). A good lotion viscosity value is less than 30,000 cPs (Ulfa et al., 2019).

Statistical Analysis

The resulting preparations were analysed using one way ANOVA and, if $p<1$, continued with the Duncan test.

RESULT AND DISCUSSION

Yield

The yield of avocado seed extract obtained with ethanol solvent at a concentration of 50 %; 70 %; and 96 % respectively yield results of 12.7 %; 22.5 %; and 21.0 %. Factors that influence the size or magnitude of the recovery of an active compound are influenced by the concentration of the filter solvent used in the extraction process (Sayuti, 2017). A concentration of 70 % can produce a higher yield value than a concentration of 50 %; this is due to the influence of chemical compounds contained in the plant, the basis of extraction is *like dissolves like* where the solubility of a compound in a solvent depends on the similarity of polarity between the solvent and the compound being extracted.

Characterization of Dry Powder Extract of Avocado Seed

The results of phytochemical screening tests on dry powder extract and avocado seed extract show that both contain alkaloids, flavonoids, tannins and saponins. The results of the water content and ash content tests can be seen in Table 2.

The results of determining the water content of avocado seed extract with ethanol solvent a concentration of 50 %, 70 %, and 96 % were at 5.99 to 7.19 %. According to the Indonesian Herbal Pharmacopoeia, content water of dry powder of seed and extracts contain no more than 10 % (Kementrian Kesehatan RI, 2022). Based on this statement, it can be stated that the results of this research meet the requirements. Water content determination testing to determine the minimum water content in the extract or dry powder which can affect the strength of the material during storage. The high-water content in the ingredients will facilitate the growth of microorganisms such as fungi which can affect the quality of dry powder and extracts (Kementrian Kesehatan RI, 2020). The ANOVA test results showed $p>0.05$, H0 was accepted, so there was no real difference in treatment (96 % ethanol, 70 % ethanol, 50 % ethanol) on the water content of avocado extract.

Determination of ash content is carried out using a furnace. This test aims to determine non-volatile components (inorganic components or mineral salts) that remain after the combustion and ignition of organic compounds. The lower the ash content of a material, the higher its purity (Kuncoro, 2015). The ANOVA test results showed $p>0.05$, H0 was accepted, so there was no significant difference in treatment (96 % ethanol, 70 % ethanol, 50 % ethanol) on the ash content of avocado extract.

Tabel 2. Result of Water Content and Ash Content

Sample Extract	Water content ± SD (%)	Ash content ± SD (%)
EtOH 50 %	6.33 ± 0.0112	5.15 ± 0.0019
EtOH 70 %	7.19 ± 0.0117	4.35 ± 0.0069
EtOH 96 %	5.99 ± 0.0128	4.19 ± 0.0013

Antioxidant Activity Value of Avocado Extracts

Antioxidant test results showed that avocado seed extract had an IC_{50} with very strong intensity. The test results can be seen in Table 3. The antioxidant test results of avocado seed extract was categorized as very strong intensity, able to inhibit free radicals by 50 %. The higher the ethanol concentration, the lower the polarity (Hanani, 2015). The strong antioxidant activity is related to the content of secondary metabolites that are extracted during the extraction process, which is in line with the percentage of yield produced. From the

yield data it can be seen that the 70 % ethanol extract has a higher yield than the 96% extract and the 50 % extract. The secondary metabolite compounds contained in avocado seed extract which are thought to have antioxidant properties are flavonoid compounds. Flavonoid compounds have potential as antioxidants because they have hydroxyl groups attached to the carbon of the aromatic ring so they can capture radicals. Flavonoid compounds will donate one hydrogen atom to stabilize the free radicals (Ningsih, 2023).

Table 3. IC50 of Avocado Seed Extract From Different Solvent Concentration

Sample extract	IC_{50} (ppm)	Intensity of antioxidant
EtOH 50 %	46.65	Very strong
EtOH 70 %	20.68	Very strong
EtOH 96 %	25	Very strong

Lotion Preparation Test Results

The extract chosen to be used as a lotion preparation is the extract that has the lowest IC_{50} value with a very strong intensity, namely 70 % ethanol extract. Next, the avocado seed extract for the lotion preparation is formulated with a 2x concentration increase for each formula. So the extract concentration used is 0.01 % in Formula 1, 0.05 % in Formula 2, and 0.5 % in Formula 3. The resulting lotion preparation can be seen in Figure 1.

The results of organoleptic and homogeneity tests can be seen in Figure 1. The organoleptic test results for all formulas have a rose aroma, the difference is the color and viscosity. The higher the concentration of the extract, the more intense the color produced. The difference in viscosity is influenced by the content of avocado seeds which have a very high fat content so avocado seeds can be used as a source of vegetable oil (Dewi et al., 2022). Overall, the formula has homogeneity. The resulting preparation must be homogeneous because it shows that the drug ingredients are evenly dispersed in the preparation. So that each part of the preparation contains the same amount of active substance (Dominica & Handayani, 2019).

The aim of the pH test is to determine the safety of the preparation used so that it does not irritate the skin. If a preparation has an acidic or low pH it can cause skin irritation. On the other hand, if the pH of a preparation is too high or alkaline it will cause the skin to become dry when used (Iskandar., et al. 2021). The pH decreases with the addition of the extract. Avocado seeds contain polyphenols (Lidi, 2020) which can cause a decrease in pH. The decrease in pH caused by lotion is 4 to 8 (Ulandari & Sugihartini, 2020).

The results of the pH quality evaluation can be seen in Table 3. The higher the concentration of avocado seed extract, the lower the pH of the preparation. The influencing factor is the increase in H^+ ions which causes the pH to become acidic (Ulandari & Sugihartini, 2020). The results of the ANOVA test show $p > 0.05$, so H0 is accepted, so there is no real difference in the treatments (sample 1, sample 2, sample 3) on the pH of the resulting lotion preparation. This can be seen in the table, where all the pH value are in the same subset.

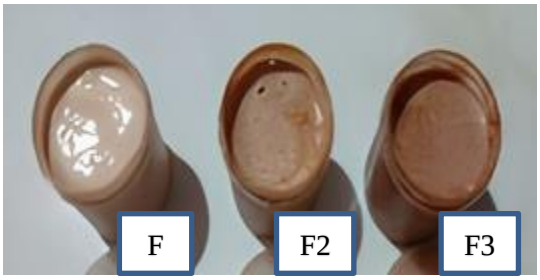

Figure 1. Avocado seed lotion
(a) F1 (0,01 %) avocado seed extract 100 mg, (b) F2 (0,05 %) avocado seed extract 500 mg, (c) F3 (0,1 %) avocado seed extract 1000 mg.

Table 3. Evaluation of pH, Spread Ability and Viscosity of Avocado Seed Lotion

Formula	pH	spread ability (cm)	Viscosity (Cps)
1	7.5285 ± SD 0.0021[a]	7.0	5088.6 ± SD 126.08[a]
2	7.4905 ± SD 0.0516[a]	6.7	5133.0 ± SD 100.00[a]
3	7.3480 ± SD 0.2913[a]	6.0	6044.3 ± SD 170.86[b]

The spread ability test of the lotion is categorized as good if it ranges between 5-7 cm (Dominica & Handayani, 2019). The higher the extract concentration, the thicker the preparation and the smaller the spread ability because the water content in the preparation is less. The viscosity test results obtained were between 5088-6044 cPs and that is categorized as good viscosity if it ranges less than 30,000 cPs (Ulfa et al., 2019). The viscosity value is directly proportional to the spreadability.

The Anova test results showed $p<0.05$, so H0 was rejected, meaning that there was a real difference in treatment (F1, F2, F3) in the viscosity value of the lotion preparation. To further explore the formula that has a significant effect, the Duncan test is carried out. Duncan test results show that the viscosity value of F1 is not significantly different from F2 but is significantly different from F3.

Antioxidant Activity Value of Lotion Preparation

The antioxidant test results of lotion avocado seed extract F3 and F2 is categorized as strong intensity and F1 is categorized as medium. The higher the extract concentration, the stronger the antioxidant activity. The IC_{50} value shows the antioxidant ability of the extract contained in the preparation. The smaller the IC 50 value, the stronger the antioxidant activity (Dewi A. T., 2019). The results of antioxidant activity tests on lotion preparations can be seen in Table 4.

Table 4. IC50 of Avocado Seed Lotion

Sampel	IC_{50} (ppm)
F1	128
F2	72
F3	55

CONCLUSION

Ethanol solvent 70 % provided the highest yield value and antioxidant activity with an IC_{50} value of 20.68 ppm. In the formulas made with 70 % ethanol extract, all three formulas meet physical quality requirements and provide IC_{50} values with moderate to strong intensity. The best formula is formula 3 with an IC_{50} value of 55 ppm.

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