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Free radical scavenging actions of virgin coconut oil

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Abstract: Natural antioxidants are quite popular in beauty products. In further product development, natural antioxidants are needed from local products of the NTB community. This study aims to optimize the antioxidant activity of local products Virgin Coconut Oil (VCO) from *Cocos nucifera* L. grown in Lombok. This type of research is experimental laboratory research using the DPPH method. VCO was isolated by conventional methods, namely standing and layered filtration. Data were obtained from the results of the UV-Vis spectroscopy test. The results showed that the free radical inhibition of VCO was strong, with an IC50 value of 51.57 and an inhibition of 48% at a 5% VCO concentration. The absorbance of DPPH decreased with increasing reaction time. The decrease in absorbance from every 5 minutes indicates the antioxidant activity continues to a stationary point. A color change indicates the stationary point to yellow. The strong antioxidant activity of VCO is used as the basis for its use as an active ingredient in various cosmetic products. VCO is also believed to have a function as a natural moisturizer.

Keywords: Natural antioxidants, Free Radical, VCO, Cosmetic.

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INTRODUCTION

Free radicals are hazardous chemical compounds that need attention for human health. Free radicals can take electrons from body cells and make new radical compounds [1]. This process occurs continuously until a non-radical compound is formed as a termination of the chemical reaction. Sources of free radicals come from within the body (endogenous) and outside the body (endogenous) [2]. The most common sources of radicals from outside the body include air pollution, UV rays, pesticides, and cigarette smoke. UV rays and exposure to cigarette smoke are sources of radicals that are difficult for humans to avoid. Especially UV rays that radiate directly to skin cells.

Skin is the outermost protective layer of the human body. Disturbances in environmental lines will first touch the skin. UV radiation causes the process of melanogenesis. The process of melanin formation in melanosomes is the oxidation of tyrosine to DOPAquinone and auto-oxidation to DOPA and DOPAchrome [3]. DOPAchrome produces eumelanin, while DOPAquinone, with the help of cysteine and glutathione, produces pheomelanin [4]. Both of these substances cause dark patches on the skin (skin pigmentation).

Skin health is a top priority for everyone. Maintaining health starts from the outer part of the body. Skin is also a social status for most people in the world. It causes the health and cleanliness of the skin to require special care and needs. Especially ingredients that can protect the skin from free radicals, namely antioxidants. Antioxidants are compounds that can bind free radicals and inhibit the oxidation process [5]. Antioxidants in the skin donate electrons to UV light free radicals, thereby inhibiting the continuous oxidant process, which affects the absence of tyrosine oxidation. The role of antioxidants is very important for human skin health. Antioxidants from extracts are widely developed in cosmetic products. One of the plants that can produce active compounds with three functions is an essential point in coconut cosmetic products (*Cocos nucifera* L.).

Virgin coconut oil (VCO) from coconut has an important role as a moisturizer and antioxidant and contains trimyristin as a whitening agent [6-7]. Virgin Coconut Oil (VCO) contains high medium-chain fatty

acids with high antioxidant activity [8]. In the body, VCO can play a role in the liver (hepatic) antioxidant defense system [9].

The dangers of free radicals must be adequately addressed. *Virgin Coconut Oil* (VCO) has acted as an antioxidant. This study aims to determine the inhibitory power of VCO against free radicals compared to ascorbic acid. This inhibitory power will be used as a reference in cosmetic formulations—especially the peel-off mask.

MATERIALS AND METHODS

This type of research is experimental laboratory research. The research variable was VCO activity with free radical inhibitory power. The test method used was the DPPH with ascorbic acid as a positive control. The research was carried out with the stages of VCO isolation and testing of free radical inhibition.

Isolation of VCO from Coconut *Cocos nucifera* L.

Isolation of VCO using a layered settling and filtering method [10-11]. Coconut flesh is grated and squeezed to produce coconut milk. Coconut milk is allowed to stand for 24 hours to separate the water, oil, and blondo. Oil is separated from water and blondo. The oil obtained was filtered using filter paper eight times.

Free Radical Inhibition Testing

1. Ascorbic Acid

25 mg of vitamin C powder (ascorbic acid) was dissolved in 25 mL of methanol to obtain a concentration of 1000 g/mL. Dilution was carried out using a pipette as much as 0.02; 0.04; 0.06; 0.08, and 0.1 mL of the mother liquor were then added to a 10 mL volumetric flask and then diluted with methanol to the limit mark. A solution with a concentration of 2 was obtained; 4; 6; 8 and 10 g/mL. Each concentration of vitamin C solution as much as 2 mL was put in a test tube and then added with 10 mL of DPPH 1000 g/mL. The solution was homogenized and incubated for 15 minutes. The absorbance of each solution was measured at a wavelength of 517 nm.

2. Virgin Coconut Oil (VCO)

The active ingredients of VCO are made in various concentrations of 1%, 2%, 3%, 4%, and 5%. Each concentration of 5 mL of sample solution was put in a test tube and then added with 10 mL of DPPH 1000 g/mL. The solution was homogenized and incubated for 15 minutes. The absorbance of each solution was measured at a wavelength of 517 nm.

3. IC50 Data Analysis

The absorbance data of each sample was used to find the % inhibition. The formula for finding the % inhibition is as follows [12].

$$\%\ \text{inhibition} \frac{\text{A blank} - \text{A sample}}{\text{A sample}}\ \text{x}\ 100\%$$

Description:
A blank = Absorbance of DPPH without sample
A sample = Absorbance of DPPH with sample
The calculation results are entered in the linear regression equation: y = ax + b
Description:
y = % inhibition
x = concentration
a = gradient
b = constant

A linear equation is used to obtain the value of IC_{50}. The IC_{50} value is obtained when the % inhibition reaches 50% of the equation y = ax+b.

$$x = \frac{50 - b}{a}$$

The price of x is IC_{50} with units of g/mL.

Table 1. The IC50 Category [13]

IC_{50} (µg/ml)	Category
x< 50	Very strong
50 ≤ x ≤100	Strong
100< x ≤150	Moderate
151 ≤ x ≤ 200	Waek

RESULTS AND DISCUSSION

Active compounds from plants are widely used in cosmetic products. Especially plants with active antioxidant compounds. The antioxidant activity comes from secondary metabolite compounds secreted by plants to adapt to their environment. The antioxidant activity developed in the peel-off mask cosmetic product is in the form of plant extracts. Due to UV radiation, secondary metabolite compounds act as proton donors to compounds or potent oxidizing agents. Neutralizing UV rays will inhibit cell damage in skin tissue [14-15]. The community or producers utilize this ability to produce a product that can protect the skin from UV rays.

Antioxidant activity does not only come from secondary metabolites. However, it can also come from fats or fatty acids [16]. Antioxidants from triglyceride fats are in the form of double bonds in the alkaline chain as proton donors (H atoms). The antioxidant activity will be more potent in the form of fatty acids. Proton donors don't just come from double bonds. The H proton can also be derived from the hydroxyl on the carboxylate functional group.

Ascorbic acid was used as a positive control in the DPPH free radical inhibition test with intense antioxidant activity. This activity is due to a large number of hydroxyl groups. This activity can be seen in the results of UV-Vis spectroscopy analysis with DPPH free radicals. Ascorbic acid with low concentration has very strong antioxidant activity with IC_{50} 49.21 (table 2). Antioxidant activity increased with increasing ascorbic acid concentration. A high concentration of ascorbic acid in percent has a very strong inhibitory power reaching 71.93%. The highest antioxidant activity was found at a concentration of 10 µg/ml, with the lowest absorbance of 0.732.

In the ascorbic acid sample, 100 L of DPPH was added. The container shows that the purple color change (DPPH) does not take long to turn yellow (figure 1). The color change indicates a reaction or process of neutralizing DPPH to DPPH2 (not radical). The reaction occurs at the concentration of ascorbic acid in percentage. The highest inhibition occurred at a 5% concentration of 93.3% (figure 2).

The antioxidant activity of VCO can be done using the DPPH method. VCO activity was compared with ascorbic acid activity against free radical inhibition. The antioxidant activity of VCO and ascorbic acid can be seen in table 3.

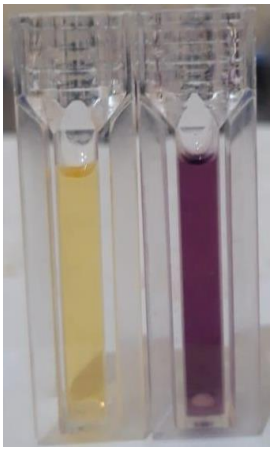

Figure 1. Color change of antioxidant activity (DPPH)

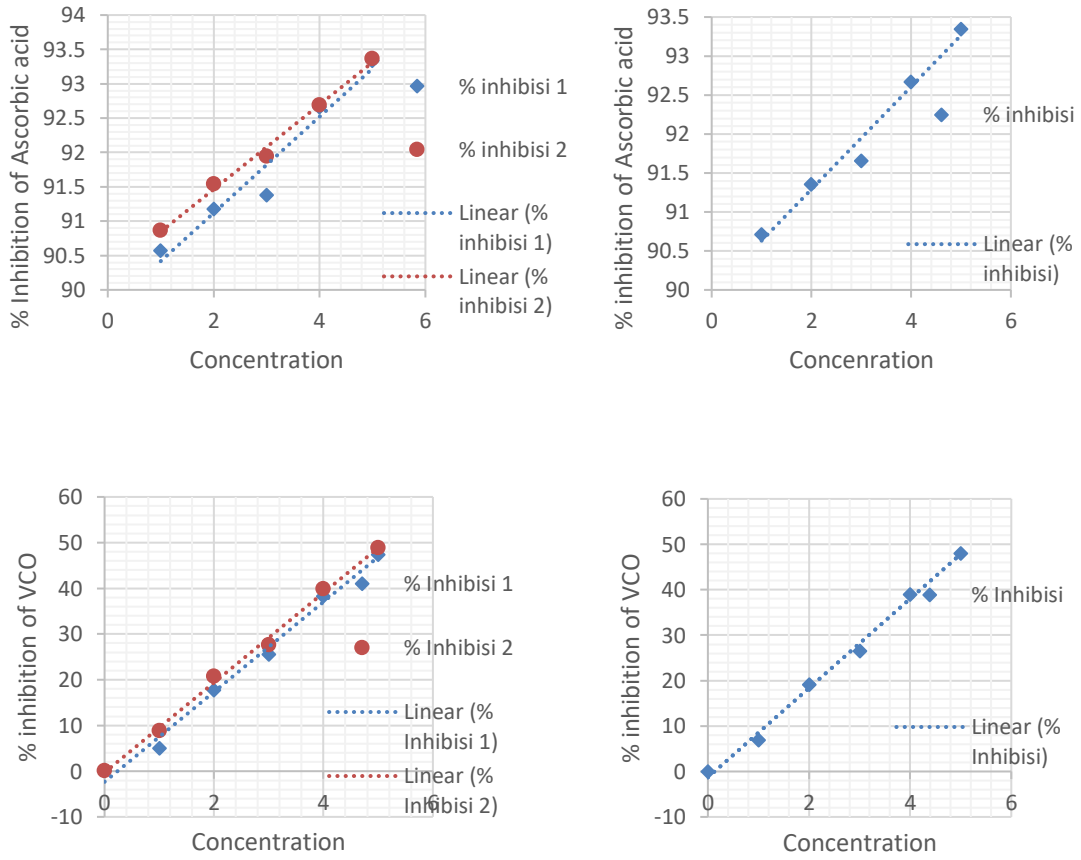

Figure 2. Linear Curve of Ascorbic Acid and VCO

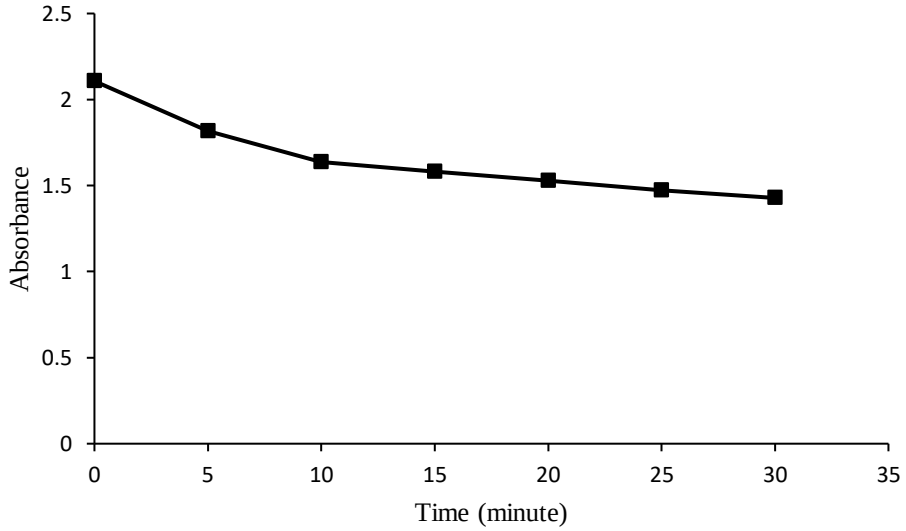

Figure 3. Antioxidant activity of VCO in unit time

Table 2. Inhibitory Power Against Free Radical

VCO			Ascorbic Acid		
Absorbance	% Inhibition	IC_{50}	Absorbance	% Inhibition	IC_{50}
1.321	0.00	51,57	2.608	0.00	49.21
1.230	6.89		2.197	15.76	
1.068	19.15		1.396	46.47	
0.970	26.53		0.978	62.50	
0.806	39.02		0.820	68.56	
0.686	48.07		0.732	71.93	

The optimization test of VCO stationary antioxidant time aims to see the activity of VCO inhibitory power at a certain time. The concentration of the sample used to formulate peel-off masks that have activity under 30 minutes. 15-30 minutes is the drying time for the peel-off mask. The 15th minute showed the maximum inhibitory activity of VCO against free radicals. The inhibitory power showed strong activity at IC50 of 51.57. The 5% concentration is used as a reference in the VCO antioxidant peel-off mask formulation. The greater the concentration of the active substance VCO, the greater the inhibitory power against free radicals, as indicated by a decrease in the concentration of free radicals in the sample (table 3). VCO has antioxidant activity with a percentage range of 37.1%-70.5% [8,17].

Apart from being an active ingredient, VCO also has a role as a moisturizer and whitener. The accumulation of melanin in facial skin results from excessive oxidation of tyrosine by solid oxidizing agents from UV rays. Tyrosine oxidation triggers the formation of eumelanin and pheomelanin. VCO can inhibit the formation of melanin on facial skin. VCO activity by inhibiting tyrosine oxidation by free radicals. Free radicals are neutralized with protons from the active ingredient VCO. The source of proton H from VCO comes from the active compound of fatty acids. The essential fatty acid in VCO is lauric acid, with a percentage of 40%. Antioxidant activity is influenced by the content of polyphenols and phenolic compounds, namely ferulic acid, p-coumaric acid [18-19], and lauric acid in the range of 48.40%-52.84% [20]. Phenolic compounds are suitable reservoir compounds for O2- and OH- from oxidation reactions that damage membranes by forming melanin [21].

Minor fatty acids also play a role in proton donors, such as linoleic acid, palmitic acid, and stearic acid. The H protons in fatty acids can come from the -OH functional group and the double bond. Oxidation reactions of unsaturated fatty acid derivatives are more effective than saturated fatty acids. The effect of the group (– $CH_2N(CH_3)_2$) tends to donate electrons to the aromatic ring so that it will give a steric impact and a more effective resonance form to form radicals that are more stable and less reactive to further oxidation reactions [22]. The number of fatty acids affects the

antioxidant activity of VCO. The action will stop when the free radical (DPPH) accepts the H atom to form $DPPH_2$, which is marked by a decrease in absorbance and a color change from purple to yellow (figure 2).

CONCLUSION

Based on the UV-Vis spectrophotometer test data, VCO has strong activity as an antioxidant with an IC50 value of 51.57. The data showed that the inhibition against free radicals increased with increasing VCO concentration. The highest activity at a concentration of 5% with a percentage of inhibition of 48.07. VCO is a natural active ingredient that is safe to use and does not cause damage to the environment in the form of waste. The molecules that make up VCO are readily biodegradable. The antioxidant activity of the compounds did not damage or interfere with the photosynthetic process of plants.

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