

Antioxidant potentials of virgin olive oil and virgin coconut oil and its cream formulation

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Abstract Exposure to ultraviolet (UV) radiation results in a photo-oxidative reaction that reduces the antioxidant defence system and increases the formation of reactive oxygen species (ROS) on the skin. Antioxidant creams protect the skin from adverse effects of UV which cause the initial signs of aging. Several creams containing 0.02 (F1); 0.2 (F2); and 2% of virgin oil were prepared. Antioxidant activities of virgin olive oil (VOO), virgin coconut oil (VCO), and the creams were evaluated by DPPH radical scavenging assay. The results showed that the oils and VOO cream formulations have antioxidant activities. Based on antioxidant activity study, VOO was selected as an active compound for the development of the antioxidant cream formulation. The EC50 values of VOO, F1, F2, and F3 of VOO were 31.7 ± 0.1 ; 176.3 ± 5.9 ; 111.9 ± 4.7 ; 90.4 ± 5.5 $\mu\text{g/mL}$, respectively. Furthermore, the EC50 value of VCO was 44.7 ± 2.0 $\mu\text{g/mL}$. The formulation of antioxidant cream containing VOO meets the requirement of physical properties and stability, except when evaluated by cycling test.

1. Introduction

Premature skin aging is a process of skin aging that is faster than normal processes. Premature skin aging is associated with excessive free radical formation. The formation of free radicals can be triggered by excessive sun exposure.

The sun's UV radiation has a beneficial effect on humans. However, this radiation also has an adverse effect according to the frequency, duration, intensity of UV radiation, and the sensitivity of the skin. Depending on the wavelength region, ultraviolet radiation is divided into 3 types namely UVA (320-400 nm), UVB (280-320 nm), and UVC (229-280 nm). The last type of radiation is very harmful to human skin. Fortunately, UV in this region is absorbed thoroughly by the ozone layer as well as large quantities of the UVB. Thus, UV radiation that reaches the earth contains approximately 5% UVB and 95% UVA. UVB directly damages DNA in cells which causes the formation of pyrimidine photoproduct, photo aging, photo carcinogenesis, and free radical [1-3].

Antioxidants are very valuable to neutralize the production of free radicals on the skin and prevent oxidative damage. When the endogenous antioxidant system is unable to neutralize the excess of free radicals, the skin needs exogenous antioxidants.

Virgin Coconut Oil (VCO) and Virgin Olive Oil (VOO) contain phenolic and tocopherol that have antioxidant activity [4-7]. VCO has received much attention because of its beneficial effects on health including reducing the risk of cancer, helping to prevent viral infections, supporting the immune system, helping to keep skin soft and smooth, not containing cholesterol, and not causing obesity [8].



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VCO was able to increase of phagocytic activity of macrophages and also be able to increase the number of lymphocytes [9].

The cream is often used in the form of cosmetic formulation. Cream preparations have several advantages, including being easily applied, being able to attach to the surface of the wear for a long time, more comfortable to use on the face, not sticky, easier to clean with water [10]. Therefore, the present study was conducted to investigate the potential use of VOO or VCO in antioxidant cream preparations.

2. Methods

2.1. Materials

Virgin olive oil (Borges) and virgin coconut oil (Al-afiat) were purchased from local stores. Cetyl alcohol and glyceryl monostearate were purchased from Cognis. Mineral oil, Tween 80, glycerin, span 80, methyl paraben, propyl paraben, distilled water were obtained from Brataco Chemica (Indonesia). Methanol was from Merck. DPPH (2,2-diphenyl-1-picrylhydrazyl) and L-ascorbic acid were purchased from Sigma Chemical Co.

2.2. Cream formulation

The ingredients used in the cream formulation are listed in Table 1 [11]. The oil phase (A) was heated at 65 °C-75 °C as well as the water phase (B). The cream is made by slowly adding ingredients A to ingredients B with rapid speed mixing to avoid the separation of oil and water phases (Ultra Turrax T25 basic IKA® - Werke). Finally, the active compounds with varying amounts of virgin oils (0, 0.02, and 2%) and Tween 80 were added into the mixture and stirred until homogeneous. The cream preparations were then stored in closed containers.

Table 1. Content of the cream formulations (% , w/v).

Ingredients	Formula			
	I	II	III	Control (-)
Ingredients A				
Cetyl alcohol (g)	9.71	9.71	9.71	9.71
Mineral oil (g)	29	29	29	29
Span 80 (g)	1.15	1.15	1.15	1.15
Glyceryl monostearate (g)	8	8	8	8
Ingredients B				
Methyl paraben (g)	0.2	0.2	0.2	0.2
Propyl paraben (g)	0.1	0.1	0.1	0.1
Glycerine (g)	7.0	7.0	7.0	7.0
Tween 80 (g)	3.29	3.29	3.29	3.29
Active compound	0.02	0.2	2	0
Distilled water (ml) qs ad to	100	100	100	100

2.3. DPPH radical scavenging assay

The DPPH solution 0.01% in methanol was used in this experiment. The maximum absorption wavelength of the DPPH solution was estimated by scanning the wavelength in the range between 400 nm and 750 nm using spectrophotometer (Shimadzu AY220). To examine the optimum reaction time, a portion of DPPH solution (0.01%, 2 mL) and the sample (30 ppm, 2 mL) were mixed and incubated 90 minutes. The absorbance of the solution was observed every 5 minutes to reach the plateau.

The determination of DPPH radical scavenging activity was carried out according to Blois [12] and modified by Brand-Williams et al. [13]. Two milliliters of the sample solution in methanol (oil or cream) was mixed with DPPH solution (2 mL, 0.01 %). After optimum reaction time at room temperature in dark conditions, the absorbance values of the sample were measured by spectrophotometer UV-Vis at the maximum absorption wavelength. The percentage of radical

scavenging activity was expressed in term of EC50 (minimum concentration needed for the sample solution to decrease the initial concentration of the DPPH by 50%). The activities were compared to L-ascorbic acid as a positive control. Methanol was used as blank [14]. The experiment was performed three times.

2.4. Physical characterization of the cream and stability test

The cream was analyzed to ensure that the formula has the desired properties such as organoleptic characteristics, pH values, spreadability, homogeneity, viscosity, and stability test.

2.4.1. *Organoleptic characteristics.* Organoleptic characteristics of the cream were evaluated by its color, odor, and texture. These characteristics were judged by visual observation. Observations are made on the newly created cream.

2.4.2. *Determination of pH.* Approximately 0.5 g of the cream formulation was weighed and dispersed in 50 ml of deionized water to determine the pH value.

2.4.3. *Homogeneity.* Homogeneity was evaluated by applying a small quantity of cream between two pieces of the object glass. The characteristic was judged by visual observation and by touch.

2.4.4. *Determination of viscosity.* Viscosity was measured by using DV-E Brookfield Viscometer with spindle No. 4 at speed 60 rpm. Approximately 25 g of cream is placed into the container. The appropriate spindle (spindle No.4) is inserted into a container. The spindle was rotated at 60 rpm value.

2.4.5. *Spreadability.* Cream (0.5 g) was placed between two pieces of circle glass plates (diameter 15 cm). The plates were pressed for 1 minute. Afterward, a weight (50 g) was applied to the upper plate for 1 minute. The spread cream diameter was measured.

2.4.6. *Stability tests.* Stability tests were performed by physical tests including thermal stability at room temperature, cycle tests, and centrifugation tests. The products stored at 25°C for a period of one month (measurements were made on day 1, week 1, week 2, week 3, and week 4). The following parameters were occupied into consideration like odor, color, homogeneity, pH, spreadability, and viscosity. The other stability test was cycling test. The cream was tested during six cycles of temperature testing. The cream was stored for 24 hours at 4 °C for, and 24 hours at 40° C. This finalizes one cycle. The color, pH, and texture were observed after each cycle. The last kind of stability test used centrifugation to predict emulsion instability. About 10 grams of creams were centrifuged for 30 minutes at 5000 rpm. The phase separation was observed after the treatment.

3. Results and discussion

DPPH radical scavenging assay has been used to evaluate the ability of VOO and VCO as antioxidants. The optimum conditions for the determination of radical scavenging activity were as follow: maximum absorption wavelength at 516 nm, the optimum reaction time was 40 minutes. In the study, we observed that VOO and VCO exhibit reactive oxygen species (ROS)-scavenging activity with 50% efficient concentration (EC50) of 31.7±0.1 and 44.7±2.0 ppm, respectively. According to observed experimental data in term of EC50, which states that the oil has EC50 less than 50µg/mL, we can categorize an antioxidant capacity of VOO and VCO into very strong antioxidant.

The antioxidant potential of VOO was higher than that of VCO as confirmed by DPPH assay. Therefore, the antioxidant potential of VOO cream may outcome in the better improvement of antioxidant-related health benefits compared with the application of VCO cream. Based on antioxidant activity study, VOO was selected as an active compound for the development of the antioxidant cream formulation. Three different concentrations of VOO were used in cream formulations. The cream

developed showed acceptable physical characteristics (Figure 1). The organoleptic characteristics, including color, odor, and texture, were tabulated in Table 2. All VOO creams had a smooth texture, homogeneous, and distinctive odor of VOO. The creams had a color that varies from white to beige white color depending on the concentration of VOO.



Figure 1. Formulated antioxidant cream of VOO.

Table 2. Evaluation of antioxidant VOO cream formulations.

	Color	Odor	Texture	Homogeneity	pH	Viscosity (cps)	Spreadability (cm)
F1	White (+++)	Characteristic of VOO	Smooth	Homogeneous	5.5	4150±2	6.0±0.3
F2	White (++)	Characteristic of VOO	Smooth	Homogeneous	5.9	4891±4	6.3±0.2
F3	White (+)	Characteristic of VOO	Smooth	Homogeneous	5.3	4681±43	6.3±0.3
(-)	White (+++)	Characteristic of glyceryl stearate	Smooth	Homogeneous	6.0	2800±2	6.0±0.3

The pH of antioxidant VOO cream formulation was found to be in range 5.3 to 5.9 which is good and recommended pH for the skin. Skin pH is normally acidic, ranging in pH values of 4–6 [15]. Changes in pH are known to cause pathogenesis of skin diseases such as dermatitis, *Acne vulgaris*, and *Candida albicans* infections. Therefore, the use of skin care products with a pH of nearly 5.5 is relevant in the prevention of the diseases [16].

The viscosities of creams were shown in Table 2. The viscosities of antioxidant VOO cream formulations were exhibited in the range 4150 to 4891 cps which indicates that the formulations have the preferred viscosity required for semisolid formulation (2000-50000 cps, SNI 16-4399-1996) [17]. There was not much variation in viscosity owing to increase in the concentration of VOO in the experimental variety of concentration (0.02-2%). However, the addition of VOO increases the viscosity of the creams.

Spreadability test was used to evaluate the ability of the cream to spread on the skin surface. The spreadability of cream preparations can be seen in Table 2. The bioavailability efficacy of the cream depends on its ability to spread [18]. Spreadability value was established to be in the range of 6.0-6.3 cm. It has resulted that the creams have desired spreadability (5-7 cm).

Stability tests were evaluated by three methods including thermal stability at room temperature, cycle tests, and centrifugation tests. The odor, texture, color, pH, spreadability, and viscosity of antioxidant VOO creams remained stable throughout the 4 weeks of stability test. Moreover, the color and pH value of creams formulation slightly changed after 6 cycles by using cycling test (Table 3). In addition, no phase separation was detected after centrifugation for 30 minutes at 5000 rpm in any of the samples.

Table 3. The color and pH value of antioxidant VOO creams at the time before and after 6 cycles.

Formula	Before Cycling Test		After 6 Cycle	
	Color	pH	Color	pH
F1	White (+++)	5.2±0.2	White (+++)	5.7±0.1
F2	White (++)	5.6±0.1	White (+)	5.5±0.1
F3	White (+)	5.4±0.1	Beige white	5.9±0.1

In the study, we observed that VOO creams can scavenge free radical species with EC50 of 176.3±5.9 ppm (F1), 111.9±4.7 ppm (F2), and 90.4±5.5 ppm (F3), respectively. The activity was less than those of positive control (L-ascorbic acid) which has an EC50 of 32.8±0.7 ppm.

4. Conclusion

The finding presented in the current study indicates that the VOO and VOO have antioxidant activity. The formulation of antioxidant cream containing VOO meets the requirement of physical characteristics. The creams were stable throughout the 4 weeks of stability test at room temperature and no phase separation was detected after centrifugation test. However, there are slightly changes after freeze-thaw conditions.

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