

The determination of antioxidants activity and sunblock *Sterculia Populifolia* extract- based cream

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ABSTRACT

Sterculia Populifolia is commonly used as the ingredient of traditional herb in Timor Island, East Nusa Tenggara, Indonesia. It contains flavonoid which roles as antioxidant. This flavonoid can be sunblock due to the existence of chromophore which commonly gives color to plants. This study aimed to determine the activity of antioxidants and sunblock through the value of Sun Protection Factor (SPF) from *Sterculia Populifolia* extract-based cream and cream preparation extract. This cream was formulated into four formulas; extract concentrate 0% (F0), 1% (F1), 3% (F2) and 5% (F3). The result showed that the whole formula of cream preparation filled the physical stability parameter. F3 cream had IC_{50} 79.179 ppm value while SPF had 5.6 value higher than F1 and F2. The smaller the IC_{50} value, the bigger antioxidant activity and SPF increased.

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Introduction

Indonesia is a country with high sun exposure in which the society mostly work outdoor and need a skin protection. The spectrum of sun exposure, ultraviolet like UVB and UVA, has bad impact on skin. UVB and UVA work synergically so that there must be a prevention and protection to reduce the impact of UVB and UVA radiation (1).

According to Bonina, the use of antioxidant on sunblock preparation can increase the photo protective activity and antioxidative essences that may prevent any disease caused by ultraviolet radiation (2). Some active compound antioxidant such as flavonoid, tannin, anthraquinone, cinnamon, vitamin C, vitamin E and beta-carotene can be used as protector toward ultraviolet (3). One of active compound sunblock in the nature is phenolic compound which include caffeic acid, ferulic acid, quercetin, apigenin, genistein, carnosic acid, silymarin, polyphenol, tannin (4).

Various plant materials can be used as the source of antioxidant and sunblock. However, *Sterculia* is hardly used because it is considered a waste. Nevertheless, this waste has high benefit such *Sterculia Populifolia*. It is including as one of *Malvaceae*, mostly found in Timor, East Nusa Tenggara province, Indonesia. The people in East Nusa Tenggara usually use it as traditional herb which is based on the knowledge and experience from generation to generations (5). Shamsudar and Paramjyothi stated that, based on the phytochemical test, *Malvaceae* contains alkaloid, flavonoid, saponin as the

main flavonoid essence that may be used as sunblock because it contains chromophore group (6). The chromophore group can absorb ultraviolet, either UVA or UVB, so that it can reduce its intensity on the skin (7). A compound that suspected of having antioxidant activity in *Sterculia Populifolia* extract is polyphenol, flavonoid, and alkaloid. Finkel & Holbrook explained that phenolic compound is able to have antioxidant activity due to its reductive characteristic (8). The main polyphenol compound such as corilagin, gallic acid, and ellagic acid have been identified as the most responsible compound toward the antioxidant activity (9). Meanwhile, flavonoid can react as antioxidant by catching free radical through giving hydrogen atom to the radical. Generally, the ability of flavonoid in catching radical depends on substitute cluster hydroxide and the ability to stabilize the phenolic radical through hydrogen or electron delocalization. Further, the phenolic radical flavonoid is stabilized by the electron delocalization which is apart from the aromatic ring. Then, the reactive oxygen will reduce the speed of propagation chained auto-oxidase reaction (10). Alkaloid compound, especially indole, quinoline, and caffeine, inside the plant was also act as hydroxyl radical reducer (11). Natural essence extracted from *Sterculia Populifolia* can be used as the source of sunblock due to its photo protective characteristic. It is in line with the reality that plants can be avoided from the sun exposure because it needs it for photosynthesis process. However, plants have self-protection mechanism to avoid damage.

From the previous explanation, plan obviously has ability to protect the skin through the essence exist within, including bioactive essence such phenolic and antioxidative essence (12). To observe the antioxidant activity, the extract can be measured through *in vitro* method, *diphenyl-1-picrylhydrazyl* (DPPH) method to find the IC_{50} . Meanwhile, the method to determine the sunblock activity of an essence is by measuring the SPF factor. Sun Protection Factor is a UV energy needed to get *Minimal Erythmal Dose* (MED) on the skin protected by a product or active sunblock essence which is compared to the amount of energy needed to get MED without protection product (13). SPF is used for UV B protection and not specifically for resisting UV A (7).

Recently, there has no study using *Sterculia Populifolia* as the material of sunblock. The existence of antioxidative flavonoid within *Sterculia Populifolia* may be used as sunblock cream to increase its benefit and affectivity. Cream formula made is the type of oil-water (m/a) because it is an optimal conduction system for flavonoid which is more acceptable, easier and more comfortable to be used on the skin rather than water-oil type (a/m).

Materials and Methods

DPPH was purchased from Merck (Germany) while vitamin C (ascorbic acid, as antioxidant standard) was purchased from Sigma Aldrich (USA). The stem bark of *Sterculia Populifolia* (c.a 10kg) was collected from a local area in Kupang, East Nusa Tenggara, Indonesia on November 2015. The plant identification was performed by Herbarium- Indonesian Institute of Science (LIPI) staff, Purwodadi, East Java, Indonesia. The ingredients of cream formula were petrolatum, mineral oil, myristate isopropyl from Merck (Germany), stearic acid, glyceryl monostearate from Franken Chemical (Germany), triethanolamine, nipagin, nipasol, and xanthan gum from Wacker Chemical (Germany).

The equipment used to do extraction was filter paper, Erlenmeyer flask, pipette, separating funnel and vacuum rotary evaporator type BUCHI Rota vapor (Switzerland). The drying tool of extract was freeze drying type Lyovapor L-300 from Buchi (Switzerland). Meanwhile, the tool used to measure the antioxidant and sunblock was Spectrophotometer UV-Vis Shimadzu. Meanwhile, the tools used to make the cream were homogenizer, Erlenmeyer, chemical glass, hot plate and thermometer.

The Extraction of Sterculia Populifolia

The fresh stem bark of *Sterculia Populifolia* (c.a 10 kg) was washed and dried under sunlight for seven days. Then, it was dried in an oven on low temperature (not more than 50 °C) to make it suitable for grinding. It was then ground to fine powder using electric grinder to obtain 3.8 kg and to transfer it to air tight container. The dried and powdered stem bark (3.8 kg) of *Sterculia Populifolia* was macerated with 70% ethanol (c.a 13 L) at room temperature. Next, it was sealed by foil and

kept for a period of 24 h with occasional shaking and stirring for three times. The whole mixture was then filtered using Buchner funnel while the filtrate was concentrated at 50 °C with a vacuum rotary evaporator and freeze dried for 24 h. The concentrated extract obtained is termed as crude extract (76 g) of a brown.

The Process of making Sterculia Populifolia extracts cream

In this study, oil-water cream was made by adding the water phase into the oil phase slowly while stirring manually and constantly crossing the clockwise until it temperature became 35 °C. The oil phase included petrolatum, mineral oil, isopropyl myristate, stearate acid, glyceryl monostearate and phenoxyethanol. Further, it was heated on water bath until 70 °C temperature. Then, the temperature was reduced to 60 °C when all ingredients were melted perfectly. The water phase included tea, xanthan gum, DMDM hydantoin and aqua dest. It was then heated on water bath until 70°C temperature. The dry extract was dissolved firstly in the aqua dest of 35 °C temperature. Then, it was added to the oil-water phase cream made and to the aqua dest until 100% of weight (100 g). Bernatonie modified the basic formula that included petrolatum (6.2 g), mineral oil (13.8 g), isopropyl myristate (1.5 g), stearate acid (7.5 g), glyceryl monostearate (5g), phenoxyethanol (0.05 g), TEA (0.2 g), xanthan gum (0.2 g), DMDM hydantoin (0.1 g), dry extract F1 1%, F2 3%, F3 5%, and aqua dest (ad 100 g) (14).

Antiradical assay

The antioxidant assay using DPPH was performed on extracts and *Sterculia populifolia* cream (F0, F1, F2 and F3). Isolated compound as well as antioxidant standard (Vitamin C) (0.3 mL: 1, 5, 10, 15 and 20 µg/mL) in methanol was mixed with methanol solution (3 mL) containing DPPH radicals (0,004%, w/w). The mixture was vigorously shaken and left to stand for 30 min in the dark before measuring the absorbance at 515 nm against a blank (14) with slight modification. Then, the scavenging ability, with 1% inhibition percentage, was calculated using eq.1.

$$\% \text{ free radical scavenging activity} = \frac{\text{blank absorbancy} - \text{sample absorbancy}}{\text{blank absorbancy}} \times 100\% \quad (1)$$

Ascorbic acid was used as positive controls. Percentage radical scavenging ability was plotted against the corresponding antioxidant substance concentration. The results were expressed as IC_{50} values and calculated by linear regression analysis of tests conducted in triplicates. The equation for the line was used to obtain the IC_{50} value defined as the amount of antioxidant substance required to 50% scavenge of free-radicals (DPPH) present in the assay system. On the other words, IC_{50} (50% inhibitory concentration)

values were obtained through extrapolation from concentration of test samples necessary to scavenge 50% of free-radicals (DPPH). A lower IC_{50} value indicates greater activity. $IC_{50} < 50\mu\text{g/mL}$ is very active; $50\mu\text{g/mL} < IC_{50} < 100\mu\text{g/mL}$ is active; $100\mu\text{g/mL} < IC_{50} < 200\mu\text{g/mL}$ is moderately active; and $IC_{50} > 200\mu\text{g/mL}$ is not active (15).

The determination of SPF value through in vitro method

The cream effectiveness was determined by determining the SPF value through *in vitro* method and UV-Vis spectrophotometry. The extraction and each cream F0, F1, F2 and F3 was weighted about ± 1.0 g. Then, it was put into a 100 mL gourd and melted by 1ml ethanol. After that, the cuvette was put into the UV-Vis spectrophotometer. The absorption and the SPF of solution melted in each preparation cream were counted. Then, it was tested three times to get accurate value and counted using equality (16).

$$\text{SPF Value} = CF \times \sum_{290}^{320} \text{Abs} \times EE \times 1$$

Where,

$EE = \text{spectrum affected from erythema}$

$I = \text{the intensity of spectrum light}$

$Abs = \text{sunblock product absorption}$

$CF = \text{correction factors (10)}$

The value of $EE \times 1$ was equal to a Constanta in which its waving length was 290-320 with 5nm for each distance (16). The value can be seen in table 1.

Table 1 value $EE \times 1$ with 290-320 waving length

Waving length (nm)	$EE \times 1$
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

The absorption was measured on 290 nm, 295 nm, 300 nm, 305 nm, 310 nm, 315 nm, and 320 nm waving length. Then, the SPF value could be found using below formula.

$$CF \times \sum_{290}^{320} \text{Abs} \times EE \times 1$$

Results

Dried extract of Sterculia Populifolia

Maceration method was used due to considering flavonoid which is susceptible toward heat causing it loses its compound (17). The liquid extract was thickened using vacuum dryer with 50°C temperature and dried up with freezer. Then, the extract obtained, 76 g, would be brown and not smelly.

The evaluation of Sterculia populifolia cream preparation

The evaluation of cream quality was conducted after the preparation was ready. The result is displayed in table 2.

1. Physical performance Test (Organoleptic)
The observation on organoleptic resulted that F0 cream without extraction was white while F1, F2 and F3 had flashy color, such as: light brown until brown because of the difference of extract concentration.
2. Acidity test (pH)
Based on 16-4399-1996 National Standard of Indonesia (SNI), Setiawan explained that pH in cream preparation was about 4.5 to 8.0 (18). This result was obtained from F1 to F3 which was 6.9, 6.5 and 6.4. Meanwhile, the value of F0 was 7.6. This result showed that the whole formula met the requirement.
3. Viscosities test
Ideal viscosities for oil-water facial cream were less than 50 dPaS (19). The test obtained 90 dPaS, 80 dPaS, 80 dPaS and 70 dPaS for F0 until F3 and three of each. The result showed that the whole formula were in accordance with viscosities cream requirement.
4. Homogenates Test
The result of homogenate test showed that the whole formula were homogeny. Rieger stated in Purwaningsih that homogenates emulsion system was influenced by mixing technique and tools used in making emulsion process (20).

Antioxidant activity

The DPPH assay is a rapid and effective colorimetric method for estimating antiradical activity. This chemical assay is widely used in natural products research to isolate phytochemical antioxidants and to test general radical absorbing capacity of extracts and pure compounds. The DPPH radical is a stable nitrogen-containing organic compound with a strong absorbance at λ_{max} 517 nm and a dark purple color. After reacting with antioxidant compounds, it is reduced and the color changes to yellow. The change can be measured by a spectrophotometer, and plotted against concentration.

Table 2 The characterizations of cream preparation

Parameter Test	Formula			
	F0 (0%)	F1 (0,5%)	F2 (3%)	F3 (5%)
Organoleptic	White	Brownish white	Light Brown	Brown
pH	7.6 ± 0.1	6.9 ± 0.2	6.5 ± 0.1	6.4 ± 0.1
Viscosities	90 ± 1.0 dPaS	80 ± 1.8 dpaS	80 ± 1.1dPaS	70 ± 0.8 dPaS
Homogenates	homogeny	homogeny	homogeny	homogeny

Table 3 Antioxidant activities of extract and vitamin C

Test samples	Linear regression	Antioxidant activity (IC ₅₀)
Extract ethanol of <i>Sterculia populifolia</i>	y= 2.608x + 6.801 (R ² = 0.998)	16.56 ± 0.47
F0	y= -0.000x + 8.564 (R ² = 0.553)	Uncountable
F1	y= 0.052x + 8.765 (R ² = 0.968)	790.15 ± 0.27
F2	y= 0.282x + 14.29 (R ² = 0.989)	126.49 ± 0.20
F3	y= 0.329x + 23.95 (R ² = 0.963)	79.17 ± 0.29
Vitamin C	y= 1.5721x + 31.962 (R ² = 0.999)	11.50 ± 0.30

In this study, the free radical scavenging activities of the extract of *Sterculia populifolia*, formula 1 cream (F1), formula 2 cream (F2), formula 3 cream (F3) and vitamin C toward the DPPH radical were determined. The good correlation was observed between the DPPH assay shown in table 1, with the regression equations were y= 2.608x + 6.801 (R² = 0.998), y= -0.000x + 8.564 (R² = 0.553), y= 0.052x + 8.765 (R²= 0.968), y= 0.282x + 14.29 (R²= 0.989), y= 0.329x + 23.95 (R² = 0.963) and y= 1.5721x + 31.962 (R² = 0.999) for *Sterculia populifolia* extract and vitamin C (figure 1). Antioxidant activity from *Sterculia populifolia* obtained IC₅₀ 16.56 ppm value. It showed that the extract of *Sterculia populifolia* antioxidant activity was very strong because IC₅₀ < 50 ppm was extremely strong (21). The test of antioxidant cream activity was done on formula 0 up to 3. From the test, it showed that F1, F2, and F3 cream obtained IC₅₀ continuously; 687.01 ppm; 126.49 ppm and 79.17 ppm. The smaller IC₅₀ value, the higher antioxidant activity increased. It meant that the extract concentration added into the cream influence the antioxidant activity of

preparation cream made. This result showed that F0 and F1 were inactive category, F2 was mediate category and F3 was strong category. This study used standard or positive control as comparative antioxidant material activity. Molyneux explained that the standard commonly used was ascorbic acid or vitamin C (15). It implied that antioxidant activity of Vitamin C was extremely active because IC₅₀ < 50 ppm was strongly active (21).

SPF of Sterculia populifolia value determination

The result of SPF value in F1 to F3 was continuously increasing from 1.7, 4.2 and 5.6. It showed that F3 had the highest SPF value because its concentration extract was the highest. The detail result can be seen in below table 4.

Discussion

The result showed that the antioxidant activity of *Sterculia Populifolia* extract using DPPH method indicated a powerful antioxidants activity score in IC₅₀

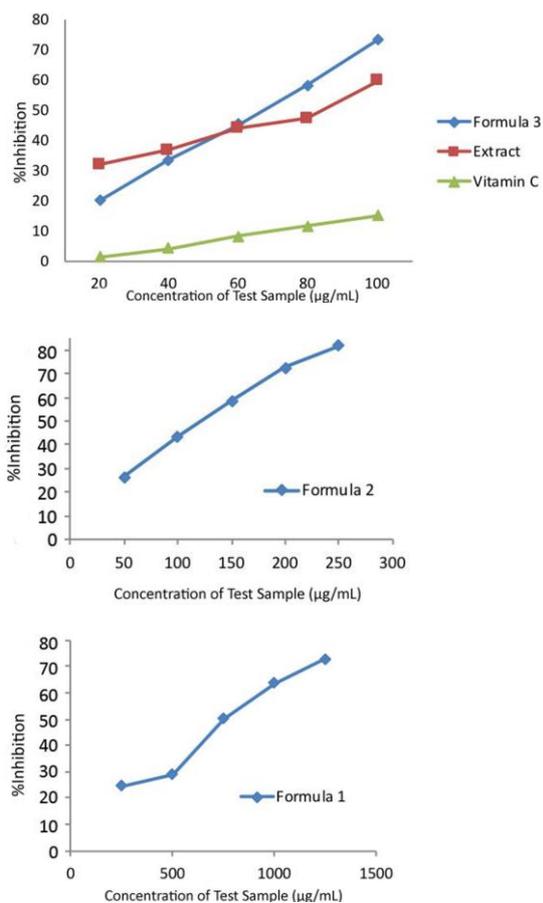


Figure 1 Free radical scavenging activity toward DPPH from extract, cream formula 1, 2 and vitamin C

Table 4 The IC_{50} and SPF

Sample	SPF Value
<i>Sterculia Populifolia</i> extract	6.88 ± 1.06
Formula 0	0.83 ± 1.09
Formula 1	1.70 ± 0.40
Formula 2	4.21 ± 0.43
Formula 3	5.61 ± 1.34

16,564. It was in accordance with Saefudin, et al result that the *Sterculia Populifolia* extract had a powerful antioxidant activity. It was suspected that the antioxidant activity from the *Sterculia Populifolia* extract occurred because the flavanoid, polyphenol, saponin, and alkaloid

compounds were a polar bioactive component, hence it dissolved in ethanol (22).

Generally, it is known that plant's phenolic compound gives a real contribution toward plant's antioxidant activity (23, 24). The most responsible polyphenol compound towards antioxidant activity is corilagin, gallic acid, and ellagic acid (9). Flavanoid is a strong antioxidant that can reduced the free radical including $O_2^{\cdot-}$, H_2O_2 , OH^{\cdot} and singlet oxygen (23). Moreover, flavanoid blocked the xanthine oxidase enzyme and damaged the superoxide activity, especially apigenin, eriodictyol, kaempferol, and luteolin (25). The phytochemical screening showed the higher relation in antioxidant activity with the amount of polyphenol compound contained inside the plant. The antioxdie activity can reached above 80% in medium until high plant's polyphenol content. Polyphenol is a basic structure of phenol (a compound with more than one hydroxyl group) that has multifunctional role as reducing agent, hydrogen donor, and radical oxigen reducer, even as a metal adhesive in some cases (24) compared to the polyphenol compound that has a higher antioxidant activity (26). Saponin has function as natural antioxidant that found many in klika. Saponin is a compound in a form of glikosida that included in natural compound with its wide function (27). The alkaloid compound, especially indole, has ability to stop the chain reaction of free radical efficiently because there is amina compound that has very long termination step. Moreover, the other alkaloid is quinoline and caffen that can be the reducer of hydroxil and melatonin radical that have important role to keep the cell from radiation (11). Saefudin et al claimed that antioxidant compound in *sterculia populifolia* is polyphenol, flavanoid, and saponin in big quantities (+++) and alkaloid in big quantities (+), which showed that klika *sterculia* has potential to share the possibilty of medicine ingredients (22). The methodology used to measure the antioxidant activity is DPPH. DPPH is a free-stable radical since the electron can be delocalized inside the molecul. The delocalized electron caused the DPPH solution inside the methanol gives the strong purple color intensity and maximum wavelenght absorption around 520 nm. DPPH contains free radical if it combined with the antioxidant extract, thus it will creates the reaction of hydrogen apprehension from the antioxidant by the DPPH free radical becomes the reduced form and decrease the intensity of the purple color (15). This color change is equal to the amount of the antioxidant activity of an ingredient in same concentration.

The experimental antioxidant activity of klika *sterculia populifolia* extract cream was done to the cream formula 1, formula 2, formula 3. The result of antioxidant activity experiment of formula 1, 2 and 3 obtained IC_{50} score sequently; 790,15 ppm; 126,49 ppm; 79,17 ppm. The smaller the score of IC_{50} , the higher the antioxidant

activity increased. It proved that the extract concentration added in the cream influences the antioxidant activity of prepared cream. The result showed the antioxidant activity of cream formula 1 was non-active antioxidant, formula 2 was medium antioxidant, and formula 3 was powerful antioxidant.

The SPF measurement was the main way to define the effectivity in sunscreen formula. The higher the SPF value, the better the UV protection would be. Sunscreen was used to help the natural body defense mechanism to protect from UV radiation. The purpose was created based on its ability in absorbing, reflecting and spreading the sunlight (28). From the experiment, *sterculia populifolia* extract was an extra SPF protection while the F1 preparation cream was the minimal SPF protection. In addition, f2 and f3 preparation cream were the medium SPF protection and f0 has no SPF score. This revealed that the basis of the cream does not influence the SPF score, the higher SPF score was purely from *sterculia populifolia* extract. In addition, the higher SPF score from *sterculia populifolia* extract and cream caused by the flavonoid compound that had potential as sunscreen because of the chromophore group. The chromophore group was a conjugated aromatic system that cause the ability in absorbing the light around the wavelength of UV light, whether in UVA or UVB.

From the experimental above, the relation between the antioxidant activity with SPF score and formula 1, 2, 3 indicated a connection. If the IC_{50} is smaller, thus the antioxidant activity is bigger and the SPF score is higher. As Widayastuti and Ariya found in their research about the antioxidant and sunscreen with strawberry leaves ethanol extract, that there was a positive relation in antioxidant and sunscreen. The bigger the antioxidant activity, the bigger the SPF score obtained (29).

After that, the last evaluation was done in *sterculia populifolia* extract formula cream to find out the stabilize preparation. The criteria observed was organoleptis test, homogenitas test, pH test and viscosity. In organoleptis test, it showed the higher concentration of *sterculia populifolia* extract made the preparation cream color browner. Whereas in homogeneity test, all the cream showed the homogeneity between basis and extract.

Further, the viscosity test was done to know the consistency of preparation cream. The viscosity in prepared cream was the endurance from a preparation to flow. The bigger the endurance, the bigger the viscosity is. The ideal viscosity for face cream in oily type inside the water was not less than 50 dPaS (19). From the examination of viscosity cream of *sterculia populifolia* extract they score obtained was 70-90 dPaS, which needed to fulfil the ideal viscosity requirements.

The pH score for all cream formula was around the pH score suitable in SNI 16-4399-1996 as a quality cream requirement and around the normal skin pH 4.5-6.5. Therefore, the produced cream is safe to be used. The pH score is important to know the acidity level from the

preparation cream so that it will not irritate the skin. If the pH is too wet, the skin can be scaly. While if the pH is contain too much acid, then the skin can be irritated. These was in accordance with Swatika, et. al result that pH 5-8 in cream is not too far from the skin's physiology, thus it can be used in skin (30).

Conclusion

From the data resulted and analyzed above, it can be concluded that *Sterculia Populifolia* extract of F1, F2, and F3 cream had IC_{50} with 16.56 ppm; 790.15 ppm; 126.49 ppm and 79.17 ppm for each. Meanwhile, the value of SPF was 1.70; 4.21; 5.61; and 6.88. Thus, antioxidant activity had correlation to SPF value. However, this study still has some limitations, such as stability test and safety test. Hence, further study, on this topic, is suggested to conduct stability and safety test to get stable and safe extract cream from *Sterculia Populifolia*.

Conflicts of Interest

The authors declare no conflict of interest.

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