ANTI-AGING PROPERTIES OF CREAM MADE WITH COCOA POLYPHENOL, ALOE VERA (*ALOE BARBADENSIS*) AND SEAWEED (*EUCHEMA COTTONI*) AS ACTIVE AGENTS

Sifat Anti-aging dari Krim Berbahan Aktif Polifenol Kakao, Aloe Vera (Aloe barbadensis) dan Rumput Laut (Euchema cottoni)

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ABSTRACT

Cream made from cocoa polyphenol combined with aloe vera and seaweed has been prepared and its anti-aging properties have been studied. Cream composition consisted of cocoa butter, olive oil, sodium dodecyl sulfate polypropylene glycol, cetyl alcohol and distilled water as the cream base. Cocoa polyphenol, aloe vera, and seaweed were used as active agents to perform anti-aging activity. Anti-aging tests were done in vivo, using male Wistar rats. Anti-aging tests included cream effectivity to protect skin from UV B radiation, which indicated by wrinkle, exfoliation, erythema and skin elasticity. The research showed that skin smeared with cream contained cocoa polyphenol, aloe vera and seaweed has less wrinkle and erythema after being exposed to the UV B lamp for 2 weeks. The cream contained 3 active ingredients (cocoa polyphenol, aloe vera and seaweed) showed less effectivity to protect against exfoliation compared to the cream with only 2 active ingredients (aloe vera and seaweed). However, the skin smeared with a cream containing 3 active ingredients is more resistant to UV B radiation than the non-smeared skin. In addition, the skin smeared with the cream containing cocoa polyphenol, aloe vera, and seaweed showed better elasticity compared to the non-smeared skin.

Keywords: anti-aging, photoaging, cocoa polyphenol, aloe vera, seaweed.

ABSTRAK

Krim berbahan polifeol kakao yang dikombinasikan dengan lidah buaya dan rumput laut telah dibuat dan dipelajari sifat anti penuaannya. Komposisi krim terdiri dari lemak kakao, minyak zaitun, sodium dodesil sulfat, polipropilen glikol, setil alkohol dan aquades sebagai basis krim. Polifenol kakao, lidah buaya dan rumput laut digunakan sebagai bahan aktif yang bertindak sebagai anti penuaan. Uji anti penuaan dilakukan secara in vivo, menggunakan tikus Wistar jantan. Uji anti penuaan yang dilakukan meliputi efektifitas krim untuk melindungi kulit dari radiasi sinar ultraviolet B, yang ditunjukkan dengan adanya kerutan, pengelupasan, kemerahan dan elastisitas kulit. Hasil penelitian ini menunjukkan bahwa kulit yang diolesi dengan krim yang mengandung polifenol kakao, lidah buaya dan rumput laut menunjukkan lebih sedikit kerutan dan kemerahan setelah dipapar dengan lampu UV B selama 2 minggu. Krim yang mengandung 3 bahan aktif (polifenol kakao, lidah buaya dan rumput laut) kurang menunjukkan efektifitas terhadap pengelupasan dibandingkan dengan krim yang hanya mengandung 2 bahan aktif (lidah buaya dan rumput laut), tetapi krim yang mengandung 3 bahan aktif tersebut masih menunjukkan proteksi yang baik terhadap kulit, dibandingkan dengan kulit yang tidak terlindungi (kulit yang tidak diolesi dengan krim apapun). Selain itu, kulit yang diolesi dengan krim yang mengandung polifenol kakao, lidah buaya dan rumput laut menunjukkan elastisitas yang lebih baik daripada yang tidak diolesi dengan krim.

Kata kunci: *anti penuaan, fotoaging, polifenol kakao, lidah buaya, rumput laut.*

INTRODUCTION

Developments of skin aging are due to a combination of time effects (intrinsic aging) and environmental effects (extrinsic aging). Both factors can affect skin simultaneously. Ultraviolet (UV) light is the major cause of extrinsic aging, following certain biological and molecular pathways. Intrinsic aging is known as skin aging or chronological aging, while extrinsic aging induced by UV light is known as photoaging (Papanagiotou, 2008).

The wrinkle is the most recognizable sign of aging. However, there are many signs of skin degradation can be associated with aging, such as pigmentation, sallowness, and dryness. Photoaging shows clinical signs include dryness, irregular, dark/ light pigmentation, sallowness, deep furrows, severe athrophy, telangiectases, premalignant, lesions, laxity, elastosis, and actinic purpura. In contrast to photoaging, chronological aging is indicated by laxity and fine wrinkling as well as the development of benign growth such as seborrheic keratosis and angiomas, but not associated with increased pigmentation or deep wrinkle shown in photoaging process (Helfrich *et al*., 2008).

The term of photoaging was firstly introduced in 1986 indicating the prolonged effect of UV light exposure on the skin (Kligman and Kligman, 1986 in Helfrich *et al*., 2008). The process of photoaging itself has been associated with protein oxidation in human skin (Sander, *et al.*, 2002). According to an *in vivo* study conducted by Sander, *et al*. (2002), depleted antioxidant enzyme expression in photodamaged skin is associated with higher level of protein oxidation. Oxidative stress also effects in the perturbation of skin barrier due to UV exposures.

These results provide a rationale for the development of efficient antioxidant strategies to prevent skin's photoaging and acute photodamage. Although sunscreens are indispensable in the prevention of skin photodamage, antioxidants in combination with sunscreens seem to be highly effective adjuncts increasing the safety and the efficacy of photoprotective products. The use of antioxidants in cosmetics has more value because it has greater protection against environmental influences (sun, pollution, wind, and temperature) on the skin, thus inhibiting aging and skin damage (Mishra and Chattopadhyay, 2010).

The aim of this research is to prepare an anti-aging cream formulated from cocoa polyphenol. Cocoa polyphenol has beensun-exposed known for its antioxidant properties to prevent cardiovascular disease (Manach *et al.,* 2005; Engler and Engler, 2011; Hooper *et al*., 2012). It was also reported in some studies (Heinrich *et al.* (2006); Williams, *et al.*, 2009) that high flavonols cocoa consumption can protect skin from harmful UV effects. However, the topical use of cocoa polyphenol are unpopular. This research uses cocoa polyphenol as a topical application with the addition of aloe vera and seaweed extract to give cooling and soothing effects on the sun-exposed skin. Such combination of the anti-aging cream product is suitable to be used in the tropical area.

METHODOLOGY

Materials and equipment

Materials used in this research were cocoa butter, non-fermented cocoa beans, aloe vera (*Aloe barbadensis*), seaweed (*Euchema cottoni*), methanol p.a.(Merck), cetyl alcohol, n-hexane p.a. (Merck), sodium dodecyl sulfate (Merck), olive oil (Bertolli), polypropylene glycol, methyl paraben, propyl paraben and distilled water.

Equipment used in this research included beaker glass, analytical balance, mixer, blender, graduated cylinder, soxhlet apparatus for polyphenol extraction, stainless steel bowl and gas stove.

Preparation of Polyphenol Extraction from Non-fermented Cocoa Beans

Cocoa beans were ground into paste using a blender. The cocoa paste was being defatted using n-hexane solvent extraction method. As much as 30 grams of cocoa paste was extracted using 300 mL of n-hexane solvent extraction for 4 hours in a soxhlet apparatus. After cocoa butter was

removed, the extraction was continued to obtain polyphenols by replacing the n-hexane solvent with methanol. Extraction with methanol was performed for 4 hours. The polyphenol gained from 1 batch of extraction process was 3.5%.

Extraction of Aloe vera and Seaweed with Hot Water

As much as 2 grams of dried seaweed was dissolved with 50 mL of hot water. Soluble fraction was used for cream preparation. As much as 2 grams of Aloe vera gel taken from fresh Aloe vera stem was dissolved in 50 mL of hot water. Soluble fraction was used for cream preparation.

Preparation of the Anti-aging Cream

Anti-aging cream was formulated referring to the previous study conducted by Khorasani (2011). The use of liquid paraffin was replaced with olive oil (in equal quantity), solid white paraffin was replaced with cocoa butter (in equal quantity) and cetearyl alcohol was replaced with cetyl alcohol, therefore increasing the amount of cetyl alcohol in anti-aging cream composition. Cocoa butter, olive oil, cetyl alcohol, sodium dodecyl sulfate and polypropylene glycol were heated in a waterbath until the cocoa butter melts. After cocoa butter melted, aloe vera and seaweed extract were added into the mixture. The mixture was then stirred to form homogeneous mixture. The resulted cream was further cooled at room temperature. Preservatives were then added to prepared cream. The preparation used was a combination of propyl paraben and methyl paraben, as much as 1% total. Two types of sample were used for the anti-aging test, Sample A: cream was not added with polyphenol and sample B: cream was added with polyphenol as much as 0.5 grams per 300 grams sample.

Testing Parameters

Testing parameters used in this research were viscosity, methanol value, α-tocopherol, pH, heavy metals (Hg, Pb, and Fe), methyl paraben, propyl paraben, microbiology testing (total plate count, *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*) and the anti-aging activity test. All quality testing of cream was conducted in Testing Unit Laboratory, Faculty of Pharmacy, Airlangga University, Surabaya. Quality testing of cream included pH test using a pH meter, viscosity with a viscometer. Determination of heavy metals content was included Hg, Pb, and Fe. Hg content was tested using ICP-AAS (Inductively Coupled Plasma-Atomic Absorbtion Spectrophotometer) method, Pb and Fe content were tested using AAS (Atomic Absorbtion Spectrophotometer). The microbiological test was referred to Pharmacopoeia IV. Methyl paraben-propyl paraben and α-tocopherol contents were determined using HPLC (High Performance Liquid Chromatography). Methanol residue was determined using GC (Gas Chromatography). The anti-aging test was completed at School of Pharmacy, Bandung Institute of Technology using healthy male Wistar rats.

Anti-aging Test

The anti-aging test was conducted at School of Pharmacy, Bandung Institute of Technology using healthy male Wistar rats as previously studied by Tsukahara *et al*., (2006). Rats were immobilized so that the posterior part of the non-hairy hind limbs are facing upwards. Prior to irradiation, the test sample was applied to the right hind limb, while the left one was received no sample treatment, and served as a control. The UVB lamp (Philip) with irradiation intensity of 10 mW/cm^2 was placed about 10 cm just above the treated skin. The irradiation was applied 5 days a week for 2 weeks, with the duration of irradiation 10-20 minutes each day. At the end of the 2^{nd} week, wrinkles on hind limbs exposed to UVB were then scored according to these following observational criteria:

0 = no rough wrinkles
1 = slightly shallow rough wrinkles
2 = some rough wrinkles
3 = some deep rough wrinkles

Along with wrinkles observations, the occurrence of skin exfoliation and erythema were also being observed and scored

according to the following observational criteria:

0 = not any
1 = a few
2 = mild
3 = many

RESULTS AND DISCUSSION

The formula of anti-aging cream used in this research was selected from a series of formulation as given in Table 1.

Table 1. Formulation of Anti-aging Cream

Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
Olive oil	2.6	1.6	1.1	0.9	2.1
Cocoa butter	3.9	2.4	1.7	1.3	3.1
Cetyl alcohol	19.5	11.8	8.5	6.6	15.5
Sodium dodecyl sulfate	3.9	2.4	1.7	1.3	3.1
Aloe vera	1.3	0.4	0.3	0.2	0.5
Seaweed	1.3	0.4	0.3	0.2	0.5
Propylene glycol	3.9	2.4	1.7	1.3	3.1
Distilled water	65	78.7	84.7	88.1	72.2

Those formulas were initial formulas to see which formula has the best characteristic. Therefore, preservatives were not added. Each formula was observed its emulsion stability and consistency. From these results, it was found that F1 cream was too thick and solid. It made F1 cream un-spreadable. Meanwhile, F2 cream was too liquid, F3 cream was sudsy and thin, and F4 was sudsy and the emulsion was broken. The F5 cream showed the best result in term of emulsion stability and consistency. The F5 cream was then used for further observation in terms of quality, anti-aging activity, and microbiology.

In order to extend the shelf life, the formulated cream was then added with preservatives from parabens class. Parabens are ester from *para*-hydroxybenzoic acid and widely used in cosmetics. Moreover, parabens are considered to be safe and low-cost preservatives. The cream then varied into two treatments: cream A with no polyphenols added, while cream B added with polyphenols. The quality of both creams was tested including pH, viscosity, heavy metals content (Pb, Hg and Fe), α-tocopherol levels, methanol content, methyl paraben, propyl paraben-and microbiology (total plate count, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*).

Cosmetic products used for human skin should have a range of pH 4-6 in order to avoid skin irritation (Jellineck, 1970). Meanwhile, determination of heavy metals content is important since heavy metals such as Pb and Hg can cause a decrease in cognitive function and kidney failure (Nnorom et al, 2005). In addition, skin exposed to heavy metals may experience rashes, discoloration, and scarring (Ladiznski et al, 2011). As this cream was also made with seaweed, the analysis of iron (Fe) content becomes necessary. Seaweed is known for the high iron source. Iron may trigger skin photodamage as iron is contributing catalytically in the oxygen radical production (Bisset, *et al*., 1991).

The compounds contained in seaweed have the antioxidant potential for applications in the cosmetic fields. Applications of these antioxidant compounds work as anti-aging, protective body cells, bleach, and UV protective (Heo and Jeon, 2009; Heo e al., 2009; Wijesinghe & Jeon, 2011). Levels of methanol testing is required because the product contains polyphenols extracted from

cocoa beans by using solvent methanol. Microbiology testing was done because *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* can cause infection when the skin are exposed (wounds).

Tabel 2. Result of Quality Analysis of Anti-aging Cream

Test Parameters	Method	Cream A	Cream B
Total Plate Count (Cfu/g)	Indonesia Farmakope IV	< 10 x 10'	< 10 x 10'
Staphylococcus aureus		Negative	Negative
Pseudomonas aeruginosa		Negative	Negative
Candida albicans		Negative	Negative
Pb (ppm)	AAS	Negative	Negative
Hg (ppm)	ICP	Negative	Negative
Fe (ppm)	AAS	[2.17 ± 0.1]	[1.79±0.1]
Methyl paraben (%)	HPLC	[0.001±0.497]	[0.0954±1.909]
Prophyl paraben (%)		[0.0633±1.97]	[0.0652±1.66]
Methanol (ppm)	GC	Negative	Negative
Alfa Tocopherol (ppb)	HPLC	Negative	Negative
pH	pH meter	[6.40 ± 0.01]	[5.78 ± 0.03]
Viscosity (dps)	Viscometer	600	300

From Table 2, it is clear that the resulted anti-aging cream has neither microbiology contamination nor heavy metals content. The cream added with polyphenol has lower pH value compared to cream that without polyphenol, as polyphenol is acidic and may lowering the pH of the cream. The pH values of both creams are 6.40 and 5.78 respectively (almost neutral). which may be associated with the use of sodium dodecyl sulfate. Methanol content in the cream is found to be negative, indicated that there is no remaining methanol residue from polyphenol extraction. The content of α-tocopherol in cream is also not found as tocopherol composition in cocoa butter as the main constituent of cream is dominated by β-tocopherol, followed by γ-tocopherol. Meanwhile, the amount of α-tocopherol in cocoa butter is not significant (Lipp and Anklam, 1997).

According to Armand (2010), the anti-aging activity was determined from a number of wrinkles caused by UV exposure. The more the wrinkle, the more ineffective the anti-aging cream is. It also indicates that the anti-aging cream has low anti-aging activity. The results of scoring calculation of wrinkles observation, exfoliation and erythema are shown in Figure 1 to Figure 5. It is shown that wrinkle was not observed in rat's hind limb treated with sample cream B. Meanwhile, hind limbs treated with sample cream A and without any cream exhibited appearance of wrinkles with score varied between 1 to 3. Hind limb treated with no cream application shows more wrinkles appearance compared to those topically treated ones. This result indicates that cream with polyphenol has better protection against wrinkles induced by UVB exposure.

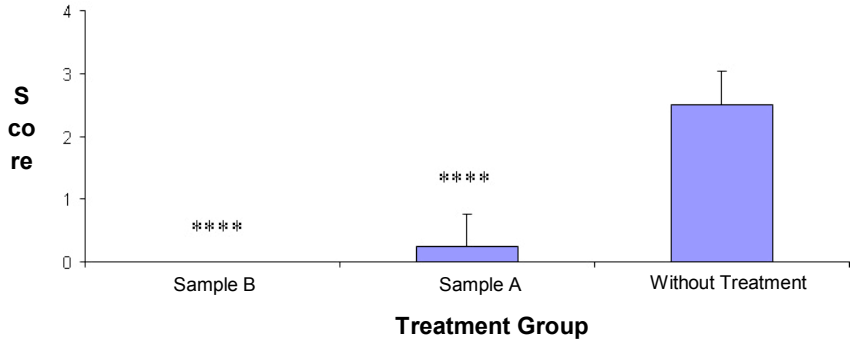


Figure 1. Effect of the sample treatment on the wrinkle visual score after the UVB exposure given 5 times a week, with duration of 10-20 minutes per day. The testing sample was applied to one hind limb before the UVB exposure.
Note: ****p<0.0001 compared to the group without treatment (ANOVA, post hoc Fisher PLSD).

Exfoliation and erythema scores are shown in Figure 2 and Figure 3. Exfoliation and erythema are less severe in hind limbs applied topically with the cream. Exfoliation scores of hind limb skin applied with sample cream A and B are lower than untreated hind limb skin as shown in Figure 2. However, the exfoliation score of the skin smeared with the cream containing polyphenol (B) is higher than that of the one smeared with the cream without polyphenol (A). It indicates that polyphenol is not effective to protect skin from exfoliation process. Application of anti-aging creams either with polyphenol or without polyphenol resulted in less erythema score on hind limb skin (Figure 3). Both creams show lower erythema scores compared to the untreated skin. However, the effectivity of cream A and cream B to protect the skin from against-erythema is only slightly different.

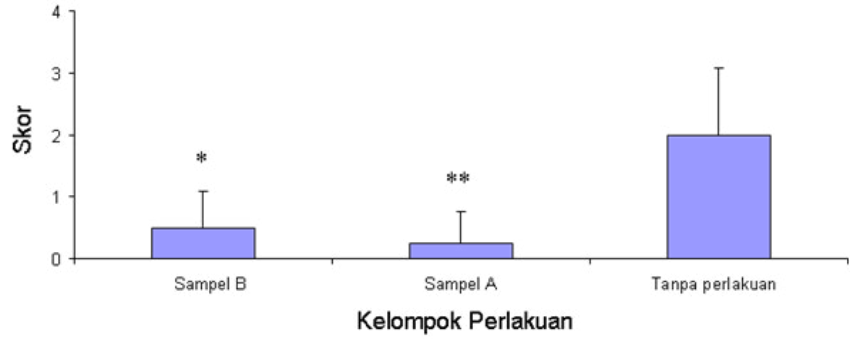


Figure 2. Effect of the sample treatment to the exfoliation score after the UVB exposure given 5 times a week, with duration of 10-20 minutes per day. The tested sample was applied to one hind limb before the UVB exposure.
Note: *p<0.05, **p<0.01 compared to the group without treatment (ANOVA, post hoc Fisher PLSD).

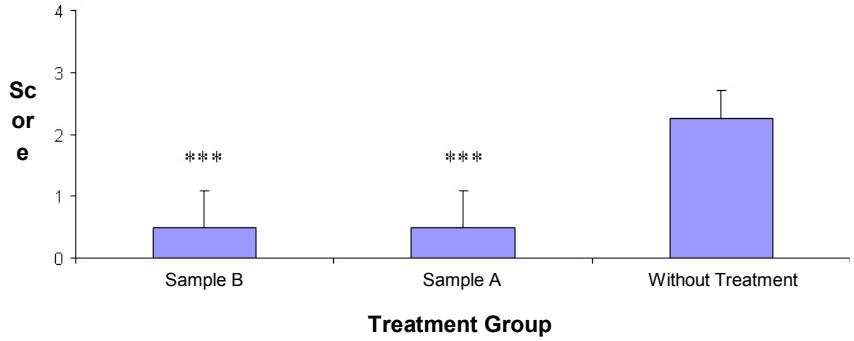


Figure 3. Effect of the sample treatment to the erythema score after the UVB exposure given 5 times a week, with duration of 10-20 minutes per day. The tested sample was applied to one hind limb before the UVB exposure.
Note: *p<0.05, **p<0.01 compared to the group without treatment (ANOVA, post hoc Fisher PLSD).

Effects of the UVB exposure and testing samples application are shown in Figure 4 and 5. Figure 4 shows the effect of UVB exposure against hind limb skin treated with sample cream B, while figure 5 shows the effect of UVB exposure against hind limb skin treated with sample cream A. On the skin applied with no sample cream, occurence of wrinkles and exfoliation are clearly observed. Meanwhile, the skins applied with sample creams, are and exfoliation are slightly observed. The skin applied with sample cream A has more wrinkles compared than that with sample test B, but shows better result in the appearance of exfoliation.

Figure 4. Effect of UVB exposure on the formation of wrinkles and exfoliations to the skin smeared with cream B. Exposure was given 10-20 minutes per day for 5 days a week during 2 weeks. *Note*: 1 and 3 = before UVB exposure, 2 = without sample, 4 = treated with sample cream B. Arrow = wrinkles; circle = exfoliation

Figure 5. Effect of UVB exposure on the formation of wrinkles and exfoliations to the skin smeared with cream B. Exposure was given 10-20 minutes per day for 5 days a week during 2 weeks. *Note*: 1 and 3 = before UVB exposure, 2 = without sample, 4 = treated with sample cream B. Arrow = wrinkles; circle = exfoliation

Chronic UVB exposure causes skin premature aging, called photo-aging. Photo-aging is characterized by mild and rough wrinkles on the skin, depigmentation, change in skin texture, loss of elasticity, and actinic precancerous keratosis (Alam dan Havey, 2010). Skin photo-aging is primarily on the degree of ultraviolet radiation (UVR) and on an amount of melanin in the skin (skin phototype). In addition, to direct or indirect DNA damage, UVR activates cell surface receptors of keratinocytes and fibroblasts in the skin, which leads to a breakdown of collagen in the extracellular matrix and a shutdown of new collagen synthesis (Pandel *et al.*, 2013). Figure 6 shows the effect of UVB exposure on skin elasticity shown by the length of UVB exposed skin.

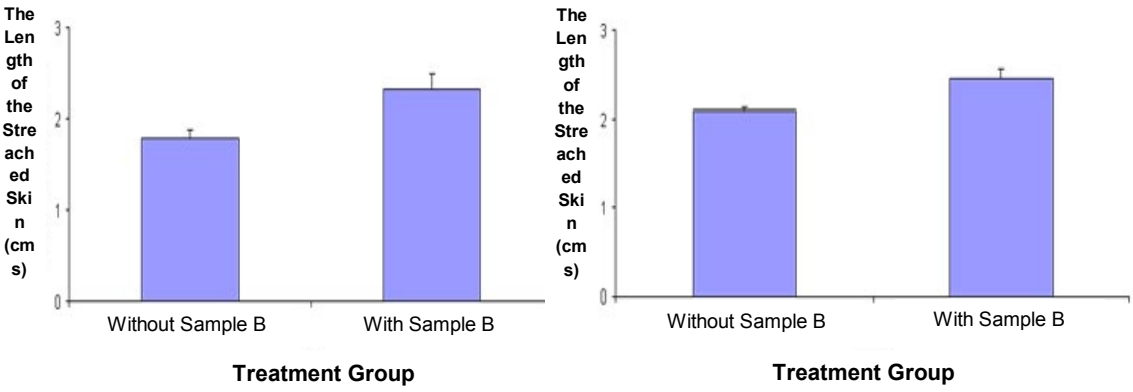
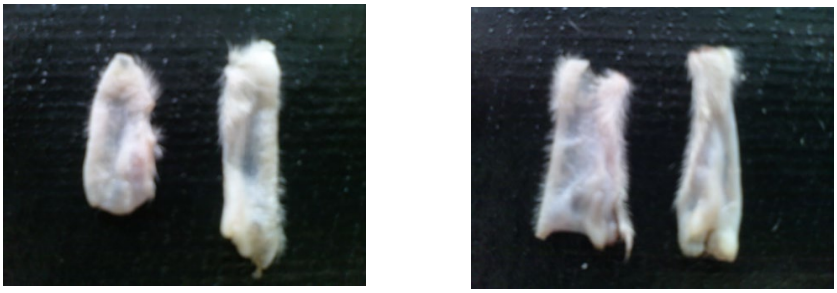


Figure 6. Effect of test samples application on rat skin length after the UVB exposure. Exposure was given 5 times a week for 2 weeks, with an exposure duration of 10-20 minutes every day. The test sample was applied to one of hind limbs before UVB exposure. After being removed from the leg, skin incision was spread maximally.

Anti-UVB often cause test results against the use of anti-aging creams (Figures 6 and 7) show that the utilization of cocoa butter and polyphenols from cocoa beans provides a protective effect against skin damage, moisturizes and protects skin elasticity. Most polyphenols are antioxidants that can neutralize free radicals that have damaging effects on cells and tissues. Free radicals are often linked to cause cell damage associated with aging. As a powerful antioxidant, polyphenols are able to inhibit the aging process. The description of the effect of UVB exposure on skin flexibility and the effect of cream sampling is shown in Figure 7.



1 2

Figure 7. Effect of test sample applied to the length of hind limbs after the UVB exposure. Exposure was given 5 times a week for 2 weeks, with an exposure duration of 10-20 minutes every day. The test sample was applied to one of the hind limbs prior to UVB exposure After being removed from the leg, skin incision wass spread maximally. *Note*: 1 = test sample ”B”, 2 = test sample ”A”, left = without sample, right = with sample

Anti-aging test shows that cream with polyphenols has the ability to inhibit the aging process, which states that the content of polyphenols in cosmetics can improve the moisture, sebum level and smoothness of the skin, also has the potential to protect skin damage that accompanies the aging process due to UVB exposure and can maintain the elasticity of the skin. The inhibitory activity of the aging process, especially wrinkles, is not caused by the presence of antioxidants in cocoa butter, olive oil, seaweed or aloe vera, as rat skin treated with sample cream B (added with cocoa polyphenol) has less wrinkles compare to the other which treated with sample cream A. Polyphenol also shows a good result in protecting skin from erythema and maintaining skin elastcity. However, polyphenol is not effective to protect skin from exfoliation.

CONCLUSION

Cream made from cocoa polyphenol combined with aloe vera and seaweed has been prepared from a composition consisted of cocoa butter, olive oil, sodium dodecyl sulfate, polypropylene glycol, cetyl alcohol and distilled water as the cream base. The best formula was achieved at the composition of 2.1% olive oil; 3.1% cocoa butter; 15.5% cetyl alcohol; 3.1% sodium dodecyl sulfate; 0.5% aloe vera; 0.5% seaweed; 3.1% propylene glycol and 72.2% distilled water. Cocoa polyphenol was then added as anti-aging. Addition of polyphenol shows satisfactory results towards several signs of aging induced by UVB exposure. The result of this research shows that skin applied with cream contained cocoa polyphenol, aloe vera and seaweed has less wrinkle and erythema after being exposed to UV B lamp for 2 weeks. Cream contained 3 active ingredients (cocoa polyphenol, aloe vera and seaweed) shows less effectivity to protect against exfoliation compare to cream with only 2 active ingredients (aloe vera and seaweed), however, cream with 3 ingredients still shows better protection to skin, compared to the unprotected skin (skin which not applied with any cream). In

addition, skin applied with cream contained cocoa polyphenol, aloe vera, and seaweed shows better elasticity, compared to those which not applied with cream.

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