Protective Effects of Acehnese Traditionally Fermented Coconut Oil (Pliek U Oil) and its Residue (Pliek U) in Ointment against UV Light Exposure: Studies on Male Wistar Rat Skin (Rattus novergicus)

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ABSTRACT

A protective effect of Acehnese traditionally fermented coconut oil (Pliek U oil) and its residue (Pliek U) have been studied against UVB radiation. The research was performed by preparing the active ingredient in ointment formulation and protective test in male Wistar rat skin (Rattus novergicus). Four different extraction methods were done to produce four active ingredients, that are Pliek U oil (PUO), ethanolic Pliek U oil extract (EPUOE), ethanolic Pliek U extract (EPUE), and ethanolic hexane residue Pliek U extract (EHRPUE). The protective effect was determined based on the erythema index (EI), melanin index (MI), and level of protein oxidation parameters. The protective level from each active ingredient was studied by PCA analysis. The result showed that the active ingredient could inhibit the redness and protein oxidation in skin tissue. PCA results show that concentration (20 v. 40%) does not affect the protection level of the active ingredients of Pliek U oil, but it does affect the one of Pliek U. Therefore, these active ingredients are potential for sunscreen application.

Keywords: Erythema index; light exposure; melanin index; PCA analysis; Pliek U

INTRODUCTION

Results from ozone depletion studies have gained serious attention, especially regarding the increase of ultraviolet (UV) radiation. This higher amount of radiation leads to an augmented risk of skin cancer (Kaffenberger et al. 2017). Experts have suggested the use of sunscreen whenever engaged in any activities where direct exposure to UV radiation cannot be avoided. Sunscreen can absorb a particular amount of radiation intensity, reducing the on-skin exposure (Hughes et al. 2013). As it offers an external body’s protection, skin protective agents are very crucial. Skin’s health determines how the body’s defense...
system works against the external threat, such as heavy metals (Irnawati et al. 2019; Suhartono et al. 2019a, 2018; Wahidah et al. 2019), pollutants (Krutmann et al. 2014), and pathogens (Ariotti et al. 2014) exposure. As a consequence, skin damage may also cause the generation of diseases such as eczema, atopic dermatitis (Suhendra et al. 2019), and skin cancer (D’Orazio et al. 2013).

As one of the body’s sensitive parts, the use of active ingredients from the natural product on sunscreen that apply on skin is the best choice owing to its higher safety, relatively low side effect (Goswami et al. 2013), and eco-friendliness (Parisioiwati et al. 2019). With the possession of tropical climate and rainforests, Indonesia is blessed with overflowing natural product resources (Zulfendi et al. 2019). In fact, the use of natural products for medical purposes has long been observed by Indonesian people, from many generations ago (Tallei et al. 2019). In the present, the said practice has been proven scientifically because of the various medical active components of the natural product (Ningsih et al. 2019) contributing to antimicrobial (Estevam et al. 2015), anti-bacterial (Nuraskin et al. 2020, 2019; Tallei et al. 2020), anti-inflammation (Amin et al. 2018), anti-oxidant (Suhartono et al. 2019b), antibiofilm (Pratiwi et al. 2015) and even anticancer (Pangastuti et al. 2016) activities.

Coconut (Cocos nucifera L.) is a tropical plant that grows and is cultivated numerously by Indonesian people (DebMandal & Mandal 2011). Coconut oil is the primary product used as a processing ingredient in culinary, beauty, and pharmaceutical products. The fatty acid contents in coconut oil are reported to have anti-oxidant properties (Nevin & Rajamohan 2010) and photo-protective (Merlin et al. 2008). Moreover, other medicinal activities of coconut oil include anti-bacterial (Kim et al. 2017; Rahmad et al. 2019), skin barrier repair (Lin et al. 2017; Vaughn et al. 2018), anti-aging, wound healing, and moisturizing in atopic dermatitis treatment (Evangelista et al. 2014; Kim et al. 2017; Lin et al. 2017).

Anti-oxidant activities of coconut oil are reported capable of reducing oxidative stress on the skin with the sun protection factor (SPF) is as high as 7.119. It is ascribed to the UV radiation absorbance by C = C (unsaturated) and C = O bonds of the fatty acid in coconut oil. This can be a basis of coconut oil used as an active sunscreen ingredient (Widiyati 2017).

In Aceh Province, coconut meat is processed through traditional fermentation techniques to yield an oil known as Pliek U oil. This process’s residue is further dried to obtain what-is-called Pliek U, which is used as an ingredient in Aceh traditional culinary (Arpi 2013).

Our newest report (Earlia et al. 2019a, 2019b) found that the Pliek U oil, Pliek U oil extract, and Pliek U extract have fatty acid contents in par with coconut oil. This finding suggested that the three traditional products are predicted to have anti-oxidant potential. In this paper, we presented the result of our investigation on the protective effects of Pliek U oil, Pliek U oil extract, and Pliek U extract on the skin of male Wistar rats (Rattus norvegicus) against UV radiation. Ointment formulation is chosen as the active ingredient medium. The protection ability is determined based on the erythema index (EI), melanin index (MI), and level of protein oxidation parameters.

**MATERIALS AND METHODS**

**PREPARATION OF PLIEK U OIL, PLIEK U OIL EXTRACT, AND PLIEK U EXTRACT**

The coconut was obtained from the raw material provider of Pliek U production house, Matang District, Bireuen Regency, Aceh Province, Indonesia. The coconut was prepared to yield a Pliek U oil (PUO) and ethanolic Pliek U oil extract (EPUOE). The active ingredients’ preparation was carried out according to the reported procedure by Earlia et al. (2019a).

Pliek U was obtained from the stated Pliek U production house. Pliek U was later prepared based on the reported procedure by Earlia et al. (2019b), to yield an ethanolic Pliek U extract (EPUE), and ethanolic hexane residue Pliek U extract (EHRPUE).

**PREPARATION OF OINTMENT FORMULATION**

Ointment formulation was prepared by mixing PUO/ EPUOE/EPUE/EHRPUE, vaseline albumin, lanolin, and m.f ointment altogether. The formulation composition is presented in Tables 1 and 2.

<table>
<thead>
<tr>
<th>No.</th>
<th>Ingredient</th>
<th>Ointment formulation (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F_0$ (control)</td>
</tr>
<tr>
<td>1</td>
<td>PUO/EPUOE/EPUE/EHRPUE</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Vaseline albumin</td>
<td>25.5</td>
</tr>
<tr>
<td>3</td>
<td>Lanolin</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>m.f ointment</td>
<td>30</td>
</tr>
</tbody>
</table>
TABLE 2. Composition of active ingredient (X) in ointment formulation

<table>
<thead>
<tr>
<th>No</th>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>P1</td>
<td>PUO</td>
</tr>
<tr>
<td>3</td>
<td>P2</td>
<td>EPUOE</td>
</tr>
<tr>
<td>4</td>
<td>P3</td>
<td>EPUE</td>
</tr>
<tr>
<td>5</td>
<td>P4</td>
<td>EHRPUE</td>
</tr>
<tr>
<td>6</td>
<td>P5</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>P6</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>P7</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>P8</td>
<td>-</td>
</tr>
</tbody>
</table>

OINTMENT PROTECTION TEST
As many as 27 male rats (Rattus novergicus) aged 8-10 weeks with an average weight of 200 g were distributed into nine groups. These groups included a group applied with an ointment without active ingredient as a control (P0), followed by P1-P4, which was administered with an ointment containing 20% PUO, EPUOE, EPUE, and EHRPUE, respectively. Meanwhile, the ointment group P5-P8 received contains 40% PUO, EPUOE, EPUE, and EHRPUE, respectively. All groups were exposed to UVB light for 24 h. The changes on physical properties were later observed. Erythema and melanin indexes were determined using a chromameter based on the method purpose by Edyson et al. (2019) and also Takiwaki et al. (2002). Afterward, the rats were terminated through a ketamine injection, and the skin is collected. The skins were homogenized with phosphate buffer (pH7.4), followed by the collection of homogenate for the determination of the protein oxidation level (Biworo et al. 2019).

FT-IR DATA PRE-PROCESSING
FT-IR spectra were transferred to Unscrambler 10.4 software for data pre-processing with smoothing, baseline correction, and standard normal variance (SNV) methods (Lasch 2012).

PRINCIPAL COMPONENT ANALYSIS (PCA)
PCA was conducted using Unscrambler 10.4 software to obtain the PCA plot. The plot was interpreted based on the grouping formed. The variable used for PCA was the infrared absorbance on each wavenumber. The analysis was conducted with several combinations of data plots. The first combination used the FT-IR data from the post-treated skin with the variation active ingredient concentration of 0, 20, and 40% to obtain the plot of PCA 1. The second combination used FT-IR data from the skin sample with a 20% concentration of an active ingredient to obtain the plot of PCA 2. The third combination used FT-IR data from the skin sample with a 40% concentration of an active ingredient to obtain a plot of PCA 3 (Wadood et al. 2019).

ETHICS APPROVAL
The ethical clearance has been obtained from the Faculty of Medicine, Universitas Syiah Kuala Banda Aceh, and General Hospital Zainal Abidin, Banda Aceh. (Approval No. 163/EA/FK-RSUDZA/2019; dated 16 July 2019).

FT-IR ANALYSIS AND PCA PREPARATION AND FT-IR ANALYSIS
As much as 1 mL, the skin homogenate was placed on an ATR crystal plate. FT-IR was operated using the absorbance method to obtain the spectrum. Specific spectra at the fingerprint wavenumber (4000-400 cm⁻¹) were observed.
RESULTS AND DISCUSSION

PROTECTIVE TEST

Investigation of the protective effects of Pliek U oil (PUO), ethanolic Pliek U oil extract (EPUOE), Pliek U extract (EPUE), and ethanolic hexane residue Pliek U extract (EHRPUE) against the UV light was determined with several parameters including erythema index (EI), melanin index (MI) and advanced oxidation protein product (AOPP) concentration. EI and MI were measured using the chromameter method resulting in L*a* b*. It states that the color cannot be red and green or yellow and blue at the same time. L* indicates a value for light, a* is a red/green coordinate, and b* is a yellow/blue coordinate. Hence, the parameter of this method can be used to identify the skin toxicity, including redness (a*), color-changing (∆E), erythema index (EI), as well as melanin index (MI).

In this study, the rats were exposed to UV radiation for 24 h, where L* a* b* values were determined later. Figure 1 presents the before and after the radiation on the rat skin.

![Figure 1](image)

**FIGURE 1.** Ratskin before and after UV radiation with ointment application. (a) Group P0 before UV radiation; (b) Group P0 after 24 h UV radiation; (c) With the ointment application containing PUO 20%; and (d) With the ointment application containing PUO 40%

In Figure 1, it is shown that the skin radiated with UV for 24 h, without the ointment application, experienced more redness compared to other groups. The redness is caused by the UV exposure allowing the vascularization to take place in the tissue. It then leads to the widening of blood vessels, causing an increase in tissue blood flow. Hemoglobin in the blood is what causing the redness in the skin to increase as well. It is corroborated by the L* a* b* values presented in Table 3.
Using the Kruskal-Wallis test to see the difference of the collective result, it was obtained that $p = 0.03$ ($p < 0.05$), indicating the presence of one or more groups giving significantly different values. Afterward, the Mann-Whitney test was employed, where group P0 is different from the other groups ($p = 0.042; p < 0.05$). Those data suggest that the ointment application with 20 and 40% active ingredients, respectively, gives an effective reduction of EI and MI.

The ointment made of Acehnese traditional coconut oil (PUO/EPUOE/EPUOE/EPUE/EHRPUE) offers a high concentration of fatty acids acting as anti-oxidants, especially the lauric acid and tocopherols. Both of the compounds can reduce the oxidative stress resulted by UV light exposure (Merlin et al. 2008; Mu’awanah et al. 2014). It is in the same agreement with Broto (2007) suggested that oral intake of virgin coconut oil (0.315 mL/100 g weight/day) for 6 weeks can improve the protection against the damage and the repairment of histopathological images on the pancreatic beta-cell of the rat after the exposure of UV light.

This study also determines the level of protein oxidation, indicated by advanced oxidation protein product (AOPP) concentration. The results can be seen in Figure 2.

### FIGURE 2. AOPP concentration given by different treatment groups

![AOPP Concentration Graph](image_url)

### TABLE 3. Values of L*a*b* given by the application of ointment with 20 and 40% active ingredient

<table>
<thead>
<tr>
<th>Sample</th>
<th>∆L*</th>
<th>∆a*</th>
<th>EI</th>
<th>IM</th>
<th>∆E</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>3.6230</td>
<td>4.5152</td>
<td>5.0517</td>
<td>3.5510</td>
<td>7.7676</td>
</tr>
<tr>
<td>P1</td>
<td>2.5887*</td>
<td>2.6807*</td>
<td>2.6703*</td>
<td>1.9462*</td>
<td>3.6104*</td>
</tr>
<tr>
<td>P2</td>
<td>4.4536*</td>
<td>2.1359*</td>
<td>2.5961*</td>
<td>1.0892*</td>
<td>3.8809*</td>
</tr>
<tr>
<td>P3</td>
<td>3.7728*</td>
<td>2.3404*</td>
<td>2.3482*</td>
<td>1.8920*</td>
<td>3.7223*</td>
</tr>
<tr>
<td>P4</td>
<td>2.9758*</td>
<td>2.4354*</td>
<td>2.3060*</td>
<td>1.7036*</td>
<td>3.2067*</td>
</tr>
<tr>
<td>P5</td>
<td>4.4881*</td>
<td>2.3707*</td>
<td>2.5700*</td>
<td>1.8899*</td>
<td>3.7973*</td>
</tr>
<tr>
<td>P6</td>
<td>2.9758*</td>
<td>2.4354*</td>
<td>2.5060*</td>
<td>1.7036*</td>
<td>3.2067*</td>
</tr>
<tr>
<td>P7</td>
<td>1.7517*</td>
<td>2.1287*</td>
<td>2.5852*</td>
<td>1.8539*</td>
<td>3.5967*</td>
</tr>
<tr>
<td>P8</td>
<td>2.7973*</td>
<td>2.1097*</td>
<td>2.6660*</td>
<td>1.7917*</td>
<td>3.5646*</td>
</tr>
</tbody>
</table>

Note: *a = significantly different with P0
From the Kruskal-Wallis test, it was obtained that \( p = 0.044 \) (\( p < 0.05 \)), indicating one group or the AOPP concentration is different. Next, the Mann-Whitney test yielded \( p = 0.046 \) (\( p < 0.05 \)), indicating that the AOPP concentration of \( P_0 \) is significantly higher than the others. Therefore, it can be concluded that the application of the ointment with 20 and 40% active ingredient may inhibit the generation of AOPP. In general, the increase in protein oxidation owing to UV exposure is proposed in Figure 3.

![FIGURE 3. AOPP concentration given by different treatment groups](image)

Based on Figure 3, UV exposure is explained as the cause of energy absorbance by the oxygen, resulting in the electronic structure change, from a triplet becoming a singlet. Singlet oxygen (radical oxygen) is an oxidant that can cause the vascularization of blood vessels allowing the tissue to be filled-in with much blood. This eventually leads to an increase of Hb concentration in the tissue, resulting in intensified redness on the tissue. Besides, singlet oxygen can also oxidize the cell membrane component protein due to the increase in protein oxidation. The administration of the ointment made of Acehnese traditional coconut oil (with 20 and 40% concentration of PUO/EPUOE/EPUE/EHRPUE) can inhibit the skin redness and protein oxidation, stem from the presence of antioxidant contents in the ointment, working in capturing the singlet oxygen.

**FT-IR ANALYSIS ON THE POST-TREATED RAT SKIN**

The effect of type and concentration of the active ingredients against the skin protection level against the UV light can be observed more specifically through FT-IR analysis of the post-treated skin. Active ingredients with a similar protection level will yield similar FT-IR spectral patterns as well; meanwhile, the difference in the pattern indicates otherwise. The similarity and difference of the FT-IR spectral patterns can be analyzed using PCA.

Figure 4 exhibits the FT-IR spectra of the post-treated rat skin, where PUO, EPUOE, EPUE, EHRPUE were used at the variation of concentrations (0, 20, and 40%). The absorbances of each functional group are observed to be relatively similar for each spectrum. Thus, it is difficult to be distinguished. All spectra, however, indicate the protein absorbance pattern in general, as reported by previous
researches. Protein structure contains carbonyl, amine, and hydroxyl groups (Martianingsih & Atmaja 2010). Puspawati et al. (2012) reported that gelatin generates identical IR absorbance of amide A at wavenumber 3600-2300 cm$^{-1}$, amide I at 1636-1661 cm$^{-1}$, amide II at 1560-1335 cm$^{-1}$, and amide III at 1300-1200 cm$^{-1}$ (Puspawati et al. 2012).

**FIGURE 4.** FT-IR spectra of post-treated rat skin using variations of type and concentration of the active ingredients from Pliek U oil and Pliek U

**PRINCIPAL COMPONENT ANALYSIS (PCA)**

The main objective of PCA is to analyze the similarity or difference between groups of the sample. The analysis product could be the sample pattern (Nadia et al. 2019) or group (Idroes et al. 2019). Plot PCA 1 (Figure 5) shows the FT-IR spectral pattern forming three groups with diversity level of 79% (71 + 8%), they are (1) Group of P0 in which the samples were treated with an ointment without active ingredient; (2) Group of P1-P6 in which the samples were applied with an ointment made of 20% active ingredient of either Pliek U or Pliek U oil (PUO 20%, EPUOE 20%, EPUOE 20% and EHRPUE 20%) and 40% active ingredient of Pliek U oil (PUO 40% and EPUOE 40%); and (3) Group P7-P8 in which the samples were given an ointment made of 40% active ingredient of Pliek U (EPUOE 40% and EHRPUE 40%). It suggests that there are differences in the protective effect level between the ointment without the active ingredient (P0) and the one with active ingredients (P1-8).

**FIGURE 5.** Plot PCA 1 of FT-IR data from the skin treated with the ointment made of active ingredients of Pliek U oil and Pliek U at 0, 20, and 40% concentrations
Based on plot PCA 1, the use of active ingredient of Pliek U oil (PUO; P1 and P5) and ethanolic Pliek U oil extract (EPUOE; P2 and P6) do not show the difference of protection level at different concentrations (20 and 40%), where their FT-IR spectral patterns are relatively similar (included in group 2). On the contrary, with the active ingredients of Pliek U (EPUE; P3 and P7) and (EHRPUE; P4 and P8), the difference of protection levels at different concentrations (20 and 40%) are observable, where the FT-IR spectral pattern is separately found in group 2 and 3. The use of active ingredient at 20% concentration also does not exhibit a significant difference in the protection level between the active ingredients from either Pliek U oil (P1-2) or Pliek U (P3-4). This is confirmed by plot PCA 2 (Figure 6) using FT-IR data of the post-treated skin with the ointment made of 20% active ingredients. It does not show any different patterns.

The difference in protection level is clearly observable for the use of 40% active ingredients. It is confirmed through plot PCA 3 (Figure 7), where the FT-IR spectral patterns of the post-treated skin with the active ingredient of Pliek U oil (P5-P6) and Pliek U (P7-P8) generate two separate groups. Nevertheless, it is worth mentioning that the groups are not separated based on their respective extraction methods, indicating that different extraction methods in this study contribute insignificantly to the level of protective effects of the ointment formulation.
CONCLUSION

Based on this study’s results, it can be concluded that the active ingredients of Acehnese traditional coconut oil (Pliek U oil) and its residue (Pliek U) possess skin protective activities against the UV light. The active ingredient can inhibit the redness and protein oxidation in skin tissue. PCA results show that concentration (20 v. 40%) does not affect the protection level of the active ingredients of Pliek U oil, but it does affect the one of Pliek U. Therefore, these active ingredients are potential for sunscreen application.

REFERENCES


