Effect of Melastoma Malabathricum L Extract on Melanine in Rats Exposed Uv Radiation.

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Abstract

UV radiation can reaches the earth's surface that can cause burning of the skin with signs such as redness of the skin (erythema), pain, blistering and peeling of the skin. UV radiation also triggers the emergence of oxidant. One of the oxidant compounds produced by UV light is hydrogen peroxide. Hydrogen peroxide can trigger the formation of tyrosine and tyrosine causes the appearance of melanine. So it is necessary to have antioxidants to overcome UV radiation. One of the plants that has antioxidant activity is M. malabathricum L. Blossoms M. malabathricum L has greater antioxidant activity than bud flowers with an IC50 value of 6.9 ppm. The purpose of this study was to analyze the protective activity against UV rays of sunscreen lotion with ethanol extract of M. malabathricum L (LSFM) flowers as seen from the erythema index, melanin, SPF value, thyrosine and skin histology of rats exposed to UV-C light. The results showed lotion extract M. malabathricum L has sun protection activity with decrease level of eritema, melanin dan hydrogen peroxide.

Keywords: Flowers Melastoma malabathricum L, erythema, melanin, Skin histology, Sun protection.

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Introduction

Since Melastoma malabathricum L is a plant that grows wild and is considered a native plant. The flower of M. malabathricum L contains quercetin and kaempferol (Isnaini, et al., 2018). Quercetin and kaempferol are flavonoid compounds that were have antioxidants activities (Dabeek and Marra, 2019). The antioxidant activity of M. malabathricum L flowers has the potential to be used as an ingredient for making sunscreen lotions. Sunscreen is a substance that contains ingredients that protect the skin against sunlight so that UV rays cannot enter the skin and can prevent skin disorders due to radiation. Sunscreen protects the skin by spreading sunlight or absorbing solar radiation energy that hits the skin, so that the radiation energy does not directly hit the skin. UV radiation can cause oxidative damage. UV light is an electromagnetic wave that can react with oxygen, causing a change in the electronic structure from a triplet to a singlet. This structure is an oxidant compound. One of the oxidant compounds produced by UV light is hydrogen peroxide. Hydrogen peroxide can trigger the formation of tyrosine and tyrosine causes the appearance of melanin. So an antioxidant compound is needed for protect skin from UV. This study aimed to analyze the activity of sunscreen lotion ethanol extract of M. malabathricum L flower seen from hydrogen peroxide, tyrosine levels, erythema index, melanin index, and skin histology of rats exposed to UV light.

Materials and Methods

Material: M malabathricum L flower, UV C lamp, 96% ethanol, cetyl alcohol, stearic acid, TEA, glycerin, liquid paraffin, prophyl paraben , methyl paraben, Oleum rosarum, aquadest

Animals: Female wistar rats with body weight 200 – 250 g were housed in independent cage with daily light and dark cycle. Food and water provided without rectriction. All procedures were accordance with our institusional guidelines and were approved by the ethics committee of faculty of medicine University of Lambung Mangkurat No. 591/KEPK-FK ULM/EC/IV/2021

Collecting of M. malabathricum L Flowers sample

Flowers of M. malabathricum L were taken from Lake Caramin, Banjarbaru City, South Kalimantan (3028'23.41"S and 114046'4.46"E). This plant was identified in the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia

Extraction of M. malabathricum L Flower

Extraction of M. malabathricum L flower using maceration method with 96% ethanol as solvent. The extraction process

was carried out 3 times. The liquid extract was evaporated using a rotary evaporator and dried using freeze drying to obtain a powder of extract M. malabathricum L flower.

Measurement SPF of extract M. malabathricum L. flower

Extract was taken 0.5 g dissolved with ethanol to make a concentration of 25 µL, 50 µL, 75 µL, 100 µL and 125 µL. The transmittance of the sample was detected in the range from 290-320 nm with a scanning wavelength of 5 nm. The SPF value is calculated using the Mansur.

Exposure Time Test

Rats was adapted for 7 days and on the 8th day, rats was shaved with an area of 15 cm2 (3 x 5 cm). After being shaved, the skin of rats was photographed, then rats was put into a UV-C cabinet with a length of 106 cm, width 34 cm and height 53 cm which contained a UV-C lamp (PHILLIPS 30 Watt). The irradiation was carried out for 5 minutes, 10 minutes, 15 minutes and 20 minutes. After irradiation, skin of rats was photographed again and the values of the erythema and melanin index were measured.

Sunscreen activity test

This research was divided into 5 groups, consisting of negative control (NC), lotion extract M. malbathricum L flower 0.006% (LSFM1), lotion extract M. malabathricum L flower 0.016% (LSFM2), lotion extract M. malabathricum L flowers 0.027% (LSFM3). Rats was put into a UV-C cabinet and photographed before and after irradiation for calculated erythema and melanin index. Then rats was anesthetized using ketamine 40 mg/kb BW intraperitoneally. Skin of the rats was taken for measurement of tyrosine, hydrogen peroxide and histopathology.

Melanin and Erythema Index Analysis

Melanin and erythema indix were measured using a chromometer based on the L * a * b* color system with the formula

Index Melanin = $1.06\Delta a^{*} 1.44\Delta L^{*}$

Index Erythema = $1{,}68\Delta a^{*} 0{,}60\Delta L^{*}$

Sample homogenization

Sample was cleaned and crushed with 1 mL of 20% TCA and 3 mL of phosphate buffer (pH 7.4). After that, it was put into a test tube and centrifuged at 2000 RPM for 10 minutes, in order to obtain a homogenate to be used for measuring H2O2 and tyrosine (Biworo et al., 2019a).

Tyrosine Analysis

Tyrosine was measured by the colorimetric method. 1 mL of sample homogenate was put into the tube, then 1 mL of 1 N NaOH solution was added and incubated in a water bath for 1 hour at 60° C. Added 15% HgSO4 and 1.5 mL of NaNO3, then vortexed.

Then it was centrifuged for 5 minutes at 1500 rpm. Measured absorbance of supernatant with a spectrophotometer at a wavelength of 470 nm.

Hydrogen Peroxide Analysis

Hydrogen peroxide was measured by the colorimeter method. Take 1 mL of sample homogenate and add 5 mL of phosphate buffer pH 7.4. Take 1 mL of the solution and added 2 mL of the dichromate solution.

The solution was heated with a water heater for 10 minutes at a temperature of 1000C. The solution was then cooled to room temperature. Then the absorbance was measured using a spectrophotometer at 570 nm (Biworo et al., 2019a).

Histology analysis

Samples were seen histologically using HE staining. Observations was made using a microscope with a magnification of 40x

Results

SPF value of the extract M malabathricum L flower is 100 ppm of the extract M. malabathricum L flower had SPF value 3.9. This is indicate that the extract M. malabathricum L has the potential as a sunscreen.

SPF value was be the basic for making the lotion of extract M. malabathricum L Flower Results of the UV irradiation test, the highest erythema index and melanin index occurred in 10 minutes of irradiation (Fig 1 and 2)

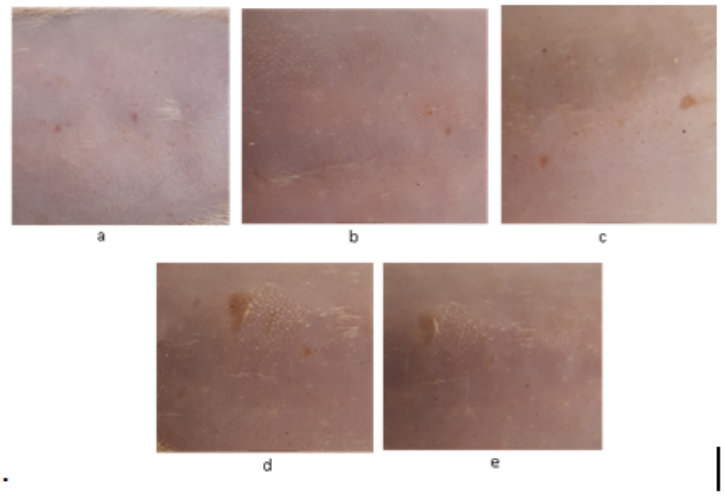

Figure 1. *Skin after exposure to UV lamp (a) 0 minutes, (b) 5 minutes, (c) 10 minutes, (d) 15 minutes, (e) 20 minutes.*

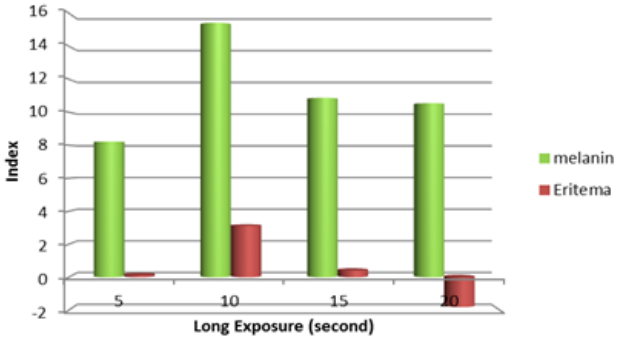

Figure 2. *Effect of long exposure to UV light on melanin and erythema index.*

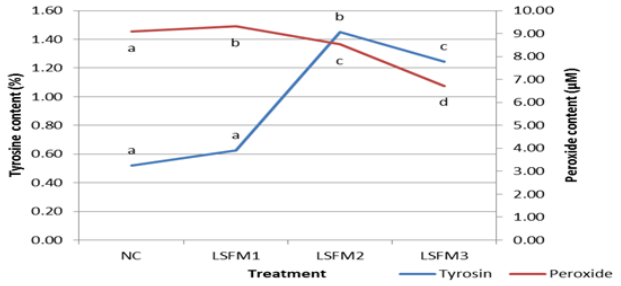

Figure 3. *Hydrogen proxide and Tyrosine levels after exposed with UV rays for 10 minutes. Significant p < 0.05*

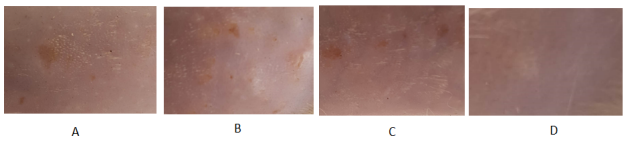

Figure 4. *Colour of Skin after exposed to UV C light for 10 minutes (a) NC, (b) LSFM 1, (c) LSFM 2, (d) LSFM 3.*

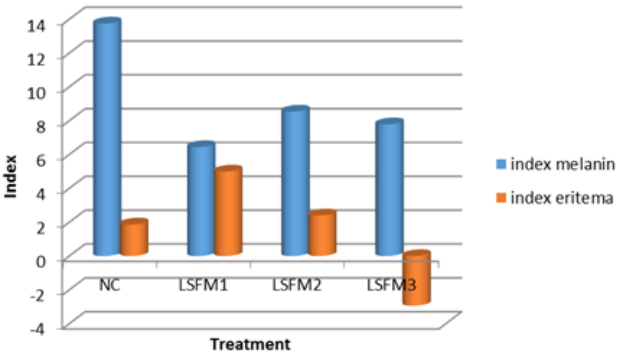

Figure 5. *Melanin and erythema index after exposed with UV rays for 10 minutes.*

Melanin index in this study was seen from the lightness of the skin. Melanin index in NC has the highest value, meaning that the application of lotion base does not cause a decrease in melanin. The LSFM1 had the lowest melanin index, meaning that giving the extract lotion caused a decrease in melanin. Melanin causes the release of histamine which contributes to sun-induced erythema. Erythema index in this study showed a reddish colour on skin. The redder skin is show the greater of erythema, and vice versa. The highest erythema index occurred in LSFM1, while the lowest occurred in LSFM3.

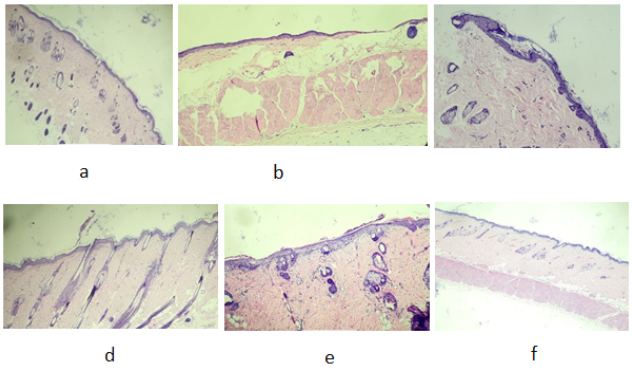

Figure 6. *Histology of skin after exposure to UV rays for 10 minutes at 40 x magnification (a) Normal skin, (b) NC (atrophic skin), (c) NC (skin bullae), (d) LSFM1 (skin bullae), (e) LSFM2 (there is slight inflammation), (f) LSFM3 (skin conditions such as normal skin).*

Histology test showed treatment with lotion of M. malabathricum L flower extract could prevent the occurrence of bullae and atrophic skin lesions Exposure to UV rays on the skin will produce reactive oxygen species (ROS). One form of ROS produced is hydrogen peroxide. Hydrogen peroxide is a compound that can induce tyrosine; tyrosine will produce dopacrome compounds which will induce melanine. So it can be said that H2O2 can induced melanine. In this research, UV radiation can induce H2O2 but concentration tyrosine still lowers. H2O2 has two activity, at lower concentration could deactivating of tyrosinase, on the other hand, at high concentrations, it causes activating tyrosinase. Tyrosinase is enzyme can induce. Inthis research show H2O2 concentration 8.53 – 9. 32 µM can induce tyrosine, but H2O2 concentration 6. 72 µM can inhibit tyrosine (LSFM3). Tyrosine induced formation of melanin. Melanin consists of 2 types, namely eumelanin (brown/black melanine) and pheomelanin (red/ blond melanine) (Slominski et al., 2004; D'orazio et al., 2013). Eumelanin is an effective UV-blocking pigment, while pheomelanin is less effective at blocking UV. The skin looks dark (black) when the melanin index is high, whereas if the erythema index is high, there is usually a possibility that more pheomelanin is formed, because erythema describes inflammation marked by a red color. But this still needs to be further research to prove this. M malabathricum L flower extract contains quercetin (Isnaini et al., 2018). Quercetin is a compound that acts as an antioxidant, anticancer and anti-inflammatory (Shin et al, 2019). In vitro, quercetin can inhibit UVC radiation-induced peroxidation in lymphosomal membranes (Svobodova et.al, 2003). Quercetin directly donates its hydrogen to scavenger ROS. Quercetin works either directly or indirectly, on several signaling pathways of oxidative stress, namely Mitogen activated Protein Kinase (MAPK), phospoinositide 3-kinase (PI3K), Akt/Protein kinase B (PKB), Keap1-Nrf2, NF-kB pathway (Wang et al, 2020). Quercetin inhibits UV-induced MMP-1. An increase MMP-1 causes an aging effect on the skin. UV light also causes skin aging through an inflammatory response, particularly COX-2

expression. The results of a study conducted by Shin et al, (2019) showed that quercetin caused a decrease in UV-induced COX-2 expression. Further research is still needed to determine the signaling pathways of sunscreen activity from M malabathricum L flower extract.

Conclusions

M malabathricum L flower can inhibit the formation of hydrogen peroxide, melanin and erythema caused by UV light exposure, but increase of tyrosine.

References

1. Amini A, Hamdin CD, Subaidah WA, et al. Effectivity of Sunscreen Cream Formulation Containing Ethanolic Extract of Wali (Brucea javanica L. Merr) Seed. Jurnal Kefarmasian Indonesia. 2019;10 (1):50-58
2. Ariani LW, E Prasetyaningrum, M Ryan Radix. Sediaan Kosmetik Shooting Gel Kombinasi Buah Jamblang (Syzgium Cumini) Dan Buah Naga (Hylocereus Undatus) Sebagai Tabir Surya Dan Indeks Iritasinya. 2020.
3. Biworo A, Atanta LW, Arianto IS, et al. Ameliorative effect of tuber extract from bawang dayak (Eleutherine palmifolia (L.) merr) against acute UV-induced skin oxidative damage in Rattus norvegicus, AIP Conference Proceedings. 2108;020010.
4. Biworo A, Abdurrahim Nupiah, N Hamidah, et al. The Effect of Dayak Onion (Eleutherine palmifolia (L.) Merr) Tuber Extract Against Erythema and Melanin Index on Rat (Rattus norvegicus) Skin Induced by Acute UV. AIP Conference Proceedings. 2108;020036.
5. Dabeek WM, Marra MV. Dietary Quercetin and Kaempferol: Bioavailability and Potential Cardiovascular-Related Bioactivity in Humans. 2019;11: 2288.
6. Isnaini, Permatasari N, Mintaroem K, et al. Analysis of quercetin and kaempferol levels in various phase of flowers Melastoma malabathricum L. International Journal of Plant Biology. 2018;9:6846.
7. Isnaini A, Yasmina H W, Nur'amin. Antioxidantd dan cytotoxicity Activity of Karamunting (Melastoma malabathricum L) Fruit Ethanolic Extract and Quercetin. Asian Pasific Journal of Cancer Prevention. 2019;20(2): 639-43.
8. Kim HE, Lee SG. Induction of ATP Synthase β by H2O2 induces melanogenesis by activating PAH and cAMP/ CREP/MITF Signaling in Melanoma Cells. The International Journal of Chemistry and Cells Biology. 2013;45:1212-1222.
9. Munoz Munoz JL, Canovas FG, Molina FG, et al. Generation of hydrogen peroxide in the melanin biosynthesis pathway. Biochimica Biophysica Acta. 2009; 29:1794-1017.
10. Pratama WA, Zulkarnain AK. Uji SPF In Vitro Dan Sifat Fisik Beberapa Produk Tabir Surya Yang Beredar Di Pasaran. 2015;11(1):275-83.
11. Shin EJ, Lee JS, Hong S, et al. Quercetin Directly Targets JAK2 and PKC and Prevents UV-Induced Photoaging in Human Skin. Int. J. Mol. Sci. 2019;20:5262.
12. Svobodova A, Psotova J, Walterova D. Natural Phenolics In The Prevention Of Up-Induced Skin Damage. A Review Biomed Papers. 2003;147(2):137-145.
13. Takiwaki H, Miyaoka Y, Kohno H, et al. Graphic analysis of the relationship between skin color change and variations in the amounts of melanin and hemoglobin. Skin Research and Technology. 2002;8:78-83
14. Wang D, Jiang Y, Sun-Waterhouse, et al. MicroRNA-based regulatory mechanisms underlying the synergistic antioxidant action of quercetin and catechin in H2O2-stimulated HepG2 cells: Roles of BACH1 in Nrf2-dependent pathways. Free Radical biology And Medicine. 2020;122-131.
15. Aslan M, Ozben T. Oxidants in Receptor Tyrosine Kinase Signal Transduction Pathways. 2003;5 (6):781-8.
16. Brenner M, Hearing VJ. The Protective Role of Melanin against UV Damage in Human Skin. Photochem Photobiol. 2008;84(3):539-49.
17. Wood JM, B Chavan I, Hafeez K U, et al. Regulation of Tyrosinase by tetrahydropteridines and H2O2. Biochemical and Biophysical Research Communications. 2004;325(4): 1412-7
18. D'Orazio J, St Jarrett, A A Ortiz, et al. UV Radiation and the Skin. Int J Mol Sci. 2013;14(6): 12222-48.

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