Asian Journal of Health Sciences 2024, 9(2):57

Open Access Full Text Article

Original Research

Torbangun (*Coleus amboinicus*) Leaves Extract Sunscreen Effect on the Melanin in the Skin Exposed to Ultraviolet-B

Jelita Pebriani Pasaribu, Chrimis Novalinda Ginting, Linda Chiuman*

ABSTRACT

Torbangun (*Coleus amboinicus*) leaves are a common medicinal plant in Indonesia, notably used by the Bataknese society as a lactagogue. Multiple studies have found that torbangun leaves are loaded with flavonoids and phenolic compounds, some of which have the potential to act as a photoprotective agent. This study aims to determine the ability of torbangun leaf extract in sunscreen cream to control the amount of melanin in the skin exposed to ultraviolet B (UVB) rays. This study is an experimental investigation employing a randomized post-test-only control group design. The subjects of this study were 28 male rats (*Rattus norvegicus*), which were divided into seven groups: the first two groups served as the negative and positive controls, while the other five groups were treated with torbangun leaf extract sunscreen cream (TLESC) at different concentrations. This study found that the application of TLESC at a concentration of 12.5% managed to control the average amount of melanin to 9.5%, whereas the base cream resulted in an average melanin content of 56.84%. There was a significant mean difference among all groups ($p<0.05$). It can be concluded that TLESC was as effective as standard sunscreen in controlling the amount of melanin in the subjects' skin.

Key words: Flavonoids, melanin, phenolics, sunscreen, torbangun

Biomedical Science Magister Program, Faculty of Medicine, Dentistry, and Health Science, Prima Indonesia University, Medan, North Sumatera, Indonesia

Correspondence

Linda Chiuman, Biomedical Science Magister Program, Faculty of Medicine, Dentistry, and Health Science, Prima Indonesia University, Medan, North Sumatera, Indonesia

Email: lindachiuman@unpri.ac.id

History

- Received: Feb 24, 2023
- Accepted: Dec 28, 2023
- Published Online: Dec 31, 2023

DOI :

https://doi.org/10.15419/ajhs.v9i2.528

Check for updates

INTRODUCTION

As a tropical country, Indonesia, which stretches along the equatorial line, has sun exposure all year round. Data from The National Aeronautics and Space Administration (NASA) Earth Observation (NEO) indicate that Indonesia has a potential for exposure to an ultraviolet (UV) index between 8 and 16[1]. The World Health Organization suggests that a UV index higher than 11 poses a high risk of causing UV damage and recommends the usage of sunscreen for a UV index higher than 2 due to the relatively high potential for solar erythema incidence after exposure[2]. One of the ultraviolet rays, ultraviolet B (UVB), plays a substantial role in vitamin D synthesis in humans. Unlike ultraviolet A from the sun, which reaches the earth's surface in its entirety, only 10% of UVB reaches the earth's surface, while the rest is reflected by the ozone layer. Despite this large proportion difference, UVB has an erythemogenic effect a thousand times more potent compared to UVA, which leads to sunburn and delayed tanning. This delayed tanning occurs due to UVB stimulating the melanocytes to synthesize melanin, hence increasing the amount of melanin in the skin[3].

Aside from all that, Indonesia also has a large multitude of medicinal plants. One such plant is torbangun (Coleus amboinicus). Known in the West as Mexican mint or Indian borage, torbangun leaves are customarily used as a lactagogue in Bataknese society, while in Ayurveda, it is oftentimes used as a remedy for respiratory, digestion, or skin conditions[4]. These multitudes of uses are due to the presence of flavonoids, alkaloids, polyphenols, tannins, glycosides, and saponins in the torbangun leaves. Multiple studies have also shown that some flavonoids and phenolic compounds have photoprotective properties, whether through antioxidant, anti-inflammatory, or other properties[5,6].

The fact that torbangun leaves contain some flavonoids and phenolic compounds that have photoprotective properties opens the possibilities of formulating torbangun leaf extract into sunscreen form.

METHODS

This study is an experimental study employing a randomized post-test-only control group design. The extraction process of Torbangun leaves and the formulation of the sunscreen cream were conducted in the Biomolecular Laboratory of Prima Indonesia University, while the experiments were conducted in the Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy at the University of North Sumatra.

BioMedPress
The Open Access Publisher

Cite this article : Pasaribu J P, Ginting C N, Chiuman L. **Torbangun (*Coleus amboinicus*) Leaves Extract Sunscreen Effect on the Melanin in the Skin Exposed to Ultraviolet-B**. *Asian J. Health Sci.* 2024; 9(2):57.

This study was conducted from August to September 2022.

Materials

The specimen used in this study was the leaves of the Torbangun plant, procured from a local farmer in North Sumatra, and identified by Herbarium Medanense at the University of North Sumatra. The Herbarium Medanense of the Faculty of Mathematics and Natural Science at the University of North Sumatra confirmed the specimen as Torbangun (Coleus amboinicus) leaves.

Animal subject

The experimental subjects in this study were rats (*Rattus norvegicus*) of the Wistar strain. The rats included in this study were male, aged between 3 to 4 months old, weighed 150-200 grams, and were healthy. The sample size for this study was calculated using the Federer formula: $(n-1)(t-1)\geq 15$, where n is the sample size and t is the number of interventions conducted. Hence, the number of samples needed for this study was 28.

The subjects were then grouped into 7 groups, each consisting of 4 subjects. These seven groups represent the 7 different interventions that the subjects received during the experiment but all received the same induction/stimulus. The first group was the negative control, while the second group was the positive control. The remaining five groups received Torbangun leaves extract sunscreen cream (TLESC) with different extract concentrations (2.5%, 5%, 7.5%, 10%, and 12.5%).

Extraction of torbangun leaves extract

The Torbangun leaves extraction process began by adding 150 grams of powdered Torbangun leaves to a container followed by 1.5 liters of 96% ethanol. This mixture was mixed continuously for 6 hours and stored in a dark chamber for 18 hours. After 18 hours, the mixture was passed through a filter, producing the first filtrate and residue. The residue was then reused for the second maceration, adding only 0.75 liters of 96% ethanol, and producing the second filtrate. Both filtrates were then mixed, and the ethanol was removed by evaporation using a rotary evaporator at a temperature of 40°C, resulting in a thick and high concentration of Torbangun leaves extract.

Phytochemical Analysis of Torbangun Leaves Extract

Phytochemical analysis was conducted in this study to determine the phytochemical contents of torbangun leaves. The analysis was conducted both qualitatively (screening) and quantitatively.

Phytochemical screening was performed to ascertain the presence of alkaloids, steroids, terpenoids, saponins, flavonoids, tannins, and glycosides in the torbangun leaves. The quantitative analysis of torbangun leaves was performed to determine the total flavonoid content and total phenolic contents.

Alkaloid Screening

Alkaloid screening in this study was carried out using four different methods/reagents: Bouchardat's, Mayer's, Dragendorff's, and Wagner's. This began by mixing 1 ml of HCl with 9 ml of aquadest and adding 500 mg of torbangun leaf extract into it. This mixture was then heated for 2 minutes in a water bath and filtered afterward to produce the filtrate. The filtrate was then divided into 4 separate test tubes, labeled A, B, C, and D. Test tube A was for Bouchardat's, B for Mayer's, C for Dragendorff's, and D for Wagner's. Into each test tube, 2 drops of its respective reagent were added. The presence of alkaloids was indicated by a yellow-orange precipitate in test tube A, a white to yellowish-white precipitate in test tube B, a reddish-blackish precipitate in test tube C, or a reddish-brown precipitate in test tube D.

Flavonoid Screening[7]

Flavonoid screening in this study was carried out using four different methods/reagents: 5% $FeCl_3$, Mg(s) + HCl(p), 10% NaOH, and H_2SO_4(p). Into four different test tubes, 2 mL of the extract was added and labeled A, B, C, and D. Into tube A, 2 drops of 5% $FeCl_3$ were added, while into tube B, a few magnesium turnings and a few drops of concentrated HCl were added. Meanwhile, into tube C, 2 mL of NaOH was added, followed by HCl. And into tube D, a few drops of concentrated H_2SO_4 were added. For tube A, the presence of flavonoids was signified by a blackish-red coloration; for tube B, it was signified by a red coloration; for tube C, after NaOH was added, an intense yellow coloration appeared, and the presence of flavonoids was signified by the dilution of the color by adding acid; and for tube D, it was signified by an orange coloration.

Steroid Screening[7]

Into 2 mL of torbangun extract, 2 mL of chloroform, and 2 mL of H_2SO_4 were added. The solution was shaken well. This resulted in the formation of a red chloroform layer and greenish-yellow fluorescence in the acid layer, indicating the presence of steroid compounds.

Triterpenoid[7]

Into 1 mL of torbangun extract, ethanol, acetic anhydride, and a few drops of H2SO4 were added. The formation of a pink to violet color indicates the presence of triterpenoids.

Saponin[7]

To the torbangun extract, 10 mL of distilled water was added and shaken vigorously to create a stable and persistent froth. If the froth persists even after adding HCl, then saponins are present.

Tannin[7]

Into the torbangun extract, a few drops of 1% $FeCl_3$ were added. The presence of brownish-green or blue-black coloration signifies the presence of tannins.

Glycoside[7]

Into a test tube, a small amount of the torbangun extract was added, followed by 2 drops of Molisch's reagent and mixed well. A few drops of H_2SO_4 were then cautiously added along the walls of the test tube to prevent mixing. The formation of a purple ring indicates the presence of glycosides.

Total Flavonoids Content[7]

500 μL of torbangun extract was added into a test tube, followed by 100 μL of 10% $AlCl_3$, 100 μL of potassium acetate (CH_3COOK), and 4.3 mL of distilled water; the mixture was then vortexed and stored at room temperature for 30 minutes. The absorbance was measured at the wavelength of 415 nm. The total flavonoid content was determined according to the standard quercetin curve and measured as milligrams of quercetin equivalent per gram (mgQE/g).

Total Phenolic Content[7]

200 μL of torbangun extract was added into a test tube followed by 1 mL of 10% Folin-Ciocalteu reagent. It was allowed to sit for one minute before adding 3 mL of Na_2CO_3. The mixture was vortexed and stored in a dark room at room temperature for two hours. The absorbance was measured at the wavelength of 760 nm. The total phenolic content was determined according to the standard gallic acid curve and measured as milligrams of gallic acid equivalent per gram (mgGAE/g).

All the phytochemical analyses were performed by an independent third party, the Organic Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences at North Sumatera University[7].

Preparation of Torbangun Leaves Extract Sunscreen Cream

The preparation of the torbangun leaves extract sunscreen cream began with separately preparing the oil and water phases. The oil phase was prepared by mixing cetyl alcohol, stearic acid, and propylparaben in a porcelain crucible on top of a water bath until completely liquefied (maintaining a stable temperature of 70-75°C). The water phase included methylparaben, half part glycerin, triethanolamine (TEA), and distilled water (aquadest), also heated in a crucible on a water bath until they were completely liquefied (maintaining a stable temperature of 70-75°C). The other half of the glycerin was used to dissolve the torbangun leaves extract. The oil and water phases were then combined in a mortar and homogenized by grinding with a pestle until a cream mass was formed. Finally, the torbangun leaves extract was added and homogenized using the same technique, resulting in the torbangun leaves extract sunscreen cream (TLESC)[7]. The cream formulation is present in **Table 1**.

Before the TLESC was used in the experiment, some physical evaluations of the cream were performed. These evaluations consisted of the organoleptic test, pH test, and spreadability, which were performed before the storage cycle test and after each cycle. The cycle consisted of storing the cream for 24 hours at a low temperature (4°C), followed by 24 hours of storage at a high temperature (40°C); this process was repeated 3 times. The organoleptic test, which uses the human senses, consists of assessing color (visual), smell (olfactory), consistency, and form (tactile).

Evaluation of Torbangun Leaves Extract Sunscreen Cream's Effect on The Amount of Melanin

The evaluation of the TLESC effect on the amount of melanin in the experiment subjects' skin was conducted after the intervention and induction phase (applying control creams/TLESC and exposing the experiment subjects to UVB) was completed.

The intervention and induction phases began by acclimatizing all experiment subjects. After that, a patch with a size of 3x3 cm was created on the back of each experiment subject by shaving it. Into that patch, interventions were carried out. In the first group, a base cream was applied, while in the second group, Vaseline® Healthy White was applied. In the other five groups, TLESC with different concentrations was applied. After the application of the interventions, all experiment subjects were exposed to UVB light for 6 hours per day for 4 weeks.

Table 1: Torbangun Leaves Extract Sunscreen Cream Formulation

Material	Base Cream	P1	P2	P3	P4	P5
Torbangun Leaves Extract*	-	2.5%	5%	7.5%	10%	12.5%
Stearate Acid	10 gr	10 gr	10 gr	10 gr	10 gr	10 gr
Acetyl Alcohol	3 gr	3 gr	3 gr	3 gr	3 gr	3 gr
Glycerin	10 gr	10 gr	10 gr	10 gr	10 gr	10 gr
TEA	2 gr	2 gr	2 gr	2 gr	2 gr	2 gr
Methyl Paraben	0.2 gr	0.2 gr	0.2 gr	0.2 gr	0.2 gr	0.2 gr
Propyl Paraben	0.05 gr	0.05 gr	0.05 gr	0.05 gr	0.05 gr	0.05 gr
Aquadest	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml

*Percentage of the total weight of the end product.

After the intervention and induction phases were completed, the experiment subjects were left without any intervention for 24 hours after the last exposure to UVB light to remove any acute UVB exposure effects. After 24 hours, a biopsy (5 mm in diameter) was taken from each experiment subject. The biopsy was then fixed by soaking it in formaldehyde phosphate for 24 hours. After that, trimming was performed to remove unnecessary parts. The tissue was then dehydrated by serially soaking it in alcohol with concentrations of 50%, 70%, 90%, 96%, and 100% for two hours at each concentration. The dehydrated tissue was then cleared by soaking it in a clearing agent (xylene) for 24 hours, which produced a transparent tissue. The transparent tissue was infiltrated twice with 60°C pure liquid paraffin for one hour each time, then embedded in liquid paraffin for 24 hours to create a paraffin block with the tissue embedded in it. The paraffin block was then serially cut using a microtome with a 3 μm thickness, and the best cut was placed on top of an object glass and incubated for 2 hours at 60°C.

The tissue slides were then stained using the Montana-Masson method to show the amount of melanin in the skin tissue of the experiment subjects. The slide was then placed under 400 times objective magnification on a microscope and photographed. The number of pixels occupied by the melanin was then counted and used to calculate the amount of melanin by the formula[8]:

Amount of Melanin = (Pixels occupied by Melanin) / (Pixels occupied by Epidermis) × 100%

Statistical Analysis

Data analysis in this study was performed using SPSS for Windows software. ANOVA with the Bonferroni post-hoc test was employed because the data of this study qualified for analysis using ANOVA (data distribution was normal and homogenous).

RESULT

Phytochemical Analysis

The phytochemical analysis of torbangun leaves, performed by the Organic Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences at North Sumatra University, found that torbangun leaves contain compounds such as alkaloids, steroids, triterpenoids, saponins, flavonoids, and tannins (**Table 2**), with total alkaloid content of 32.36 mgQE/gram (milligram quercetin equivalent per gram) and total phenolic content of 88.98 mgGAE/gram (milligram gallic acid equivalent per gram).

Physical Evaluation of Sunscreen Cream Formulation

The physical evaluation of sunscreen cream formulations found that all formulations were stable, even after undergoing a cycle test. The organoleptic test demonstrated that all formulations retained their color, smell, form, and homogeneity after each cycle. The pH of all cream formulations, before the cycle test, was within the safe range for skin usage, and after each cycle, the pH decreased but remained within the safe range for skin usage. While remaining stable, the spreadability of the sunscreen cream formulation increased after the cycling test.

Evaluation of TLESC Effect on the Amount of Melanin

The evaluation of the effect of TLESC on the amount of melanin in the experiment subject's skin found that the first experimental group subject had the highest amount of melanin, 56.84%, while the lowest amount

Table 2: Qualitative Phytochemical Analysis

Compounds	Reagent	Result
Alkaloid	Bouchardath	+
	Mayer	+
	Dragendroff	+
	Wagner	+
Steroid	Salkowsky	-
Triterpenoid	Lieberman-Burchad	+
Saponin	Aquadest + Alkohol 96%	+
Flavonoid	$FeCl_3$ 5%	-
	Mg(s) + HCl(p)	-
	NaOH 10%	-
	H_2SO_4(p)	+
Tanin	$FeCl_3$ 1%	+
Glikosida	Mollisch	-

Table 3: Average Amount of Melanin Based on The Intervention

Group	Average Amount of Melanin
Base Cream (Negative Control)	56.84%
Vaseline® Healthy White (Positive Control)	8.76%
TLESC 2.5%	39.59%
TLESC 5.0%	31.28%
TLESC 7.5%	21.38%
TLESC 10.0%	13.78%
TLESC 12.5%	9.50%

TLESC: Torbangun leaves extract sunscreen cream

of melanin was found in the second group, which received Vaseline® Healthy White, with 8.76% melanin. Meanwhile, TLESC has shown differing performance, where the amount of melanin decreased with an increase in TLESC concentration. The 2.5% TLESC average amount of melanin was 39.59%, while the highest concentration, TLESC 12.5%, had an average amount of melanin of only 9.5% (**Table 3**).

Data normality and homogeneity of variance tests performed on the study's data found that the data were normally distributed ($p>0.05$) and homogeneous ($p>0.05$), hence qualifying for ANOVA.

ANOVA carried out on the study's data found that there was a significant mean difference in the amount of melanin between all the groups. Further post-hoc (Bonferroni) tests found that there was a significant mean difference between the negative control group and the rest of the groups ($p<0.05$). A similar significant mean difference was also found in the TLESC 2.5%, 5%, and 7.5% groups compared to other groups. However, the TLESC 10% did not show a significant mean difference compared to the TLESC 12.5% ($p>0.05$), while showing a significant mean difference compared to the other groups ($p<0.05$). The TLESC 12.5% was the only formulation to show no significant difference compared to the positive control and TLESC 10% ($p>0.05$), while showing a significant difference compared to the other groups ($p<0.05$).

DISCUSSION

Phytochemical screening of torbangun leaves in this study found that the leaves contain alkaloids, steroids, triterpenoids, saponins, flavonoids, and tannins (**Table 2**). This result is in accordance with mul-

tiple studies of the phytochemical profiles of torbangun leaves[9–12]. The study by Matita et al. showed that torbangun leaves contained flavonoids, phenols, and tannins, while Damanik *et al.* found that alkaloids, triterpenoids, saponins, tannins, and flavonoid compounds were present in the ethanol extract of torbangun leaves[9,10]. Meanwhile, the studies by Laila *et al.* and Gomes *et al.* focused on the phenolic and flavonoid content of torbangun leaves[11,13]. Different extraction and detection methods performed in these studies contribute to the varying results in the phytochemical profile, both qualitatively and quantitatively. Ethanol extraction, which underwent a fractionation process, can produce three different extract fractions: hexane, ethyl acetate, and the aqueous fraction. For instance, in ethanol extracts, qualitative tests detected the presence of alkaloids, flavonoids, triterpenoids, saponins, and tannin compounds[9]. However, in the hexane fraction, tannins and flavonoids were not detected, while in the ethyl acetate fraction, saponins and tannins were not detected[9]. Aside from different extraction methods, different detection techniques among the studies also affected the results. For example, the Laila et al. study determined the phytochemical content of torbangun leaves using Gas Chromatography-Mass Spectrometry (GC-MS), and the Gomes et al. study used High-Performance Liquid Chromatography (HPLC)[11,13]. This study, however, was carried out using the maceration technique with ethanol as the solvent, and phytochemical detection was conducted both qualitatively and quantitatively.

Several studies have reported comprehensive phytochemical profiles of torbangun leaves[14–16]. Those studies support the findings in this study that the ethanol extract of torbangun leaves contains multiple compounds, such as alkaloids, steroids, triterpenoids, saponins, flavonoids, and tannins[14–16]. In this study, flavonoid detection was carried out using four different methods, but only one managed to detect the presence of flavonoids. However, this result can be accepted as confirming the presence of flavonoid compounds in torbangun leaves. This conclusion was supported by the fact that quantitative analysis of torbangun leaves found a total flavonoid content of 32.36 mgQE/gram. This total flavonoid content was slightly lower than that found by Taher et al., which was 41.4 mgQE/gram, and much lower compared to the findings of Ślusarczyk et al., which were 96.83 ± 1.4 mgQE/gram[14,17].

Apart from total flavonoid content, this study also quantified the total phenolic content of torbangun leaves. The study found that the total phenolic content of torbangun leaves was 88.89 mgGAE/gram. The Ślusarczyk *et al.* study found that phenolic compounds were the major phytochemical content in torbangun leaves with total phenolic contents of 112.95 ± 0.88 mgGAE/gram[14]. A review article by Rahmawati et al. found that generally, the total phenolic content of torbangun leaves was more than 40 mgGAE/gram, with the highest concentration reaching 313 mgGAE/gram, which was obtained from the hot water extract of torbangun leaves[15].

The differences in total flavonoid and total phenolic content can be attributed to different extraction techniques, as well as the geographic and climatic conditions where the torbangun plant grows. A study by Gomes *et al.* found that the total flavonoid and total phenolic content of torbangun varied every month of the year, with the lowest total flavonoid content of 19.34 mgQE/gram in February and the highest total flavonoid content of 49.82 mgQE/gram in December, and the lowest total phenolic content of 79.41 mgGAE/gram in February and the highest total phenolic content of 164.7 mgGAE/gram in July[12]. A study by Ślusarczyk *et al.* that compared the phytochemical profile of torbangun cultivated in Indonesia and Poland found that torbangun leaves cultivated in Indonesia had lower total flavonoid and total phenolic content compared to those cultivated in Poland[14]. The Ślusarczyk *et al.* study found that torbangun leaves cultivated in Indonesia had a total flavonoid content of 13.47 mgQE/gram and a total phenolic content of 23.61 mgGAE/gram, while the torbangun leaves cultivated in Poland had a total flavonoid content of 96.83 mgQE/gram and a total phenolic content of 112.95 mgGAE/gram[14].

This study aims to understand the potency of torbangun leaves extract formulated into cream form in controlling the amount of melanin in the skin of experiment subjects exposed to UVB light. This study found that, even though at lower concentrations (2.5% ~ 10%), TLESC performed better than the base cream in controlling the amount of melanin in the subjects' skin, 12.5% TLESC was the only concentration that performed as well as Vaseline® Healthy White, the positive control (**Table 3**). This finding showed that TLESC has a photoprotective effect, with a better effect at higher concentrations. Rosmarinic acid and caffeic acid are likely responsible for this photoprotective effect[13].

Rosmarinic acid, aside from having photoprotective properties, also has anti-radical free properties and is effective as a lipid peroxide antagonist[18]. The study by Sanchez-Campillo et al. found that oral administration of rosmarinic acid showed photoprotective effects in experiment subjects exposed to UVA

light for 120 minutes per session with 100 total sessions [19]. Seventy percent of the subjects given rosmarinic acid did not have even slight dysplasia on the skin and the rest 30% only had slight dysplasia, while 80% of the subjects not given rosmarinic acid had moderate skin dysplasia, and the rest had severe skin dysplasia [19]. The same study surmised that this photoprotective effect of rosmarinic acid was due to its antioxidant and anti-inflammatory properties [19]. Another study by Cândido *et al.* found that rosmarinic acid had a cytoprotective effect against UVB radiation-induced oxidative stress [18]. The mechanism behind these properties is yet to be explained, though the inhibition of reactive oxygen species (ROS) induced by UVB radiation and inflammation marker, along with intracellular signaling pathways inhibition [20]. Another explanation of this melanin inhibition was proposed by Oliveira et al., which hypothesized that rosmarinic acid, at higher concentrations, acts as a competitive inhibitor of the tyrosinase binding site, leading to the formation of o-quinone derivatives and blocking the enzymatic oxidation of L-DOPA to o-dopaquinone, while its antioxidant action reduces o-dopaquinone, limiting the synthesis of dopachrome, hence melanin [21]. Multiple studies also showed that rosmarinic acid was the most prevalent phenolic compound in torbangun leaves, followed by caffeic acid [12,14,16,22].

Caffeic acid, a phenolic compound, was the second-highest phenolic compound in torbangun leaves after rosmarinic acid. Like rosmarinic acid, caffeic acid also has photoprotective properties, especially when combined with ferulic acid, which is also present in torbangun leaves [14,15,23,24]. Caffeic acid can protect the skin from UVB light, and ferulic acid is a strong UVB absorber, which is why the combination of the two has a significant photoprotective effect [23]. An in-vitro experimental study by Maruyama *et al.* found that both ferulic and caffeic acids decreased melanogenesis in a melanoma cell line [25]. The same study also found that ferulic acid and caffeic acid inhibit the conversion of tyrosine to DOPA, and DOPA to dopaquinone, which ultimately inhibits melanogenesis [25]. Ferulic acid also inhibits melanogenesis by directly binding itself to tyrosinase, while direct tyrosinase binding was not observed with caffeic acid [25].

The study by Terto *et al.* found that torbangun leaves have a sun protective factor (SPF) of 12.6 [13]. The same study also concluded that torbangun leaves extract has high potency as a material for sunscreen to protect skin from damage by UVA and UVB exposure [13]. The findings of Terto *et al.* support this study's result that TLESC, especially at higher concentrations, can control the amount of melanin in the experiment subjects' skin exposed to UVB light. In the Bonferroni post-hoc test, 12.5% TLESC showed no significant mean difference with Vaseline® Healthy White, the positive control ($p>0.05$). This means that 12.5% TLESC performed as well as Vaseline® Healthy White in controlling the amount of melanin in the subjects' skin.

CONCLUSIONS

This experimental study was carried out to understand the efficacy of torbangun leaf extracts as a sunscreen cream in controlling the amount of melanin in the skin. The study concluded that torbangun leaf extract sunscreen was effective at preventing the increase of melanin in the skin due to UV exposure, especially at a high concentration (12.5%). The study also found that torbangun leaves contain alkaloids, steroids, triterpenoids, saponins, flavonoids, and tannins, with total flavonoid contents of 32.36 mgQE/gram and total phenolic content of 88.89 mgGAE/gram. The findings in this study, supported by several previous studies, also suggest that torbangun leaves have photoprotective properties, which make them a good candidate for sunscreen material. This is particularly true at higher concentrations, attributed to its high flavonoid and phenolic contents.

ABBREVIATIONS

ANOVA - Analysis of Variance
$FeCl_3$ - Ferric Chloride
GC-MS - Gas Chromatography-Mass Spectrometry
H_2SO_4 - Sulfuric Acid
HCl - Hydrochloric Acid
HPLC - High-Performance Liquid Chromatography
mgGAE/g - Milligrams of Gallic Acid Equivalent per Gram
mgQE/g - Milligrams of Quercetin Equivalent per Gram
NaOH - Sodium Hydroxide
NASA - The National Aeronautics and Space Administration
NEO - Earth Observation
ROS - Reactive Oxygen Species
SPF - Sun Protection Factor
SPSS - Statistical Package for the Social Sciences
TEA - Triethanolamine
TLESC - Torbangun Leaves Extract Sunscreen Cream

UV - Ultraviolet
UVA - Ultraviolet A
UVB - Ultraviolet B

ACKNOWLEDGMENTS

None.

AUTHOR'S CONTRIBUTIONS

All authors significantly contributed to this work, read and approved the final manuscript.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study and its protocols have been approved by the Ethics Committee of Health Study of Prima Indonesia University as stated in letter No. 056/KEPK/UNPRI/IV/2022 and declared in accordance to seven WHO 2011 Standards.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. NASA Earth Observations. UV Index [Internet]. NASA Earth Observation; 2010 [cited 2022 Jan 10]. Available from: https://neo.gsfc.nasa.gov/view.php?datasetId=AURA_UVI_CLIM_M;.
2. World Health Organization. Global Solar UV Index: A Practical Guide. Geneva: World Health Organization Press; 2002.;.
3. Rünger TM. Cutaneous Photobiology. In: Kang S, Amagai M, Bruckner AL, editors. Fitzpatrick's Dermatology. 9th ed. New York: McGraw-Hill; 2019.;.
4. Chevallier A. Encyclopedia of Herbal Medicine. 3rd ed. New York: DK Publishing; 2016.;.
5. Forero AG, Mantilla DAV, Nunez LA, Ocazionez RE, Stashenko EE, Fuentes JL. Photoprotective and antigenotoxic effects of the flavonoids apigenin, naringenin, and pinocembrin. Photochemistry and Photobiology. 2019;95(4):1010-8. doi: 10.1111/php.13085.;.
6. Irianti T, Nanda T, Sulaiman S, et al. Pembuatan sediaan tabir surya ekstrak etanol rimpang temu kunci (Boesenbergia pandurata (Roxb.) Schlecht), aktivitas inhibisi fotodegradasi tirosin dan kandungan fenolik totalnya. Majalah Farmasi. 2020;16(2):218-32. doi: 10.22146/farmaseutik.v16i2.49421.;.
7. Egbuna C, Ifemeje JC, Maduako MC, et al. Phytochemical Test Methods: Qualitative, Quantitative and Proximate Analysis. In: Phytochemistry, Volume 1: Fundamentals, Modern Techniques, and Applications. Waretown (NJ): Apple Academic Press Inc.; 2019.;.
8. Fithria RF, Anas Y, Putri FAW. The antihyperpigmentation effect of pare leaves (Momordica charantia L.) ethanol extract on guinea pig (Cavia porcellus) skin. Jurnal Ilmiah Cendekia Eksakta. 2017;2(1):42-53. doi: 10.3194/ce.v2i1.1797.;.
9. Damanik RM, Kustiyah L, Hanafi M, Iwansyah AC. Evaluation lactogenic activity of ethyl acetate fraction of torbangun (Coleus amboinicus L.) leaves. IOP Conference Series: Earth and Environmental Science. 2017;101(1):012007. doi: 10.1088/1755-1315/101/1/012007.;.
10. Matita IC, Mastuti TS, Maitri S. Antioxidant properties of different types of torbangun herbal tea. Reaktor. 2020;20(1):18-25. doi: 10.14710/reaktor.20.1.18-25.;.
11. Laila F, Fardiaz D, Yuliana ND, Damanik MRM, Dewi FNA. Phytochemical contents of torbangun (Coleus amboinicus Lour) from fractionation of pressurized liquid extraction. Jurnal Ilmu Pertanian Indonesia. 2020;25(2):224-31. doi: 10.18343/jipi.25.2.224.;.
12. Gomes J de M, Terto MVC, Do Santos SG, da Silva MS, Tavares JF. Seasonal variations of polyphenols content, sun protection factor and antioxidant activity of two Lamiaceae species. Pharmaceutics. 2021;13(1):110. doi: 10.3390/pharmaceutics13010110.;.
13. Terto MVC, Gomes JM, Araújo DIAF, et al. Photoprotective activity of Plectranthus amboinicus extracts and HPLC quantification of rosmarinic acid. Revista Brasileira de Farmacognosia (Brazilian Journal of Pharmacognosy). 2020;30(2):183-8. doi: 10.1007/s43450-020-00040-6.;.
14. Ślusarczyk S, Cieślak A, Yanza YR, et al. Phytochemical profile and antioxidant activities of coleus amboinicus lour. Cultivated in Indonesia and Poland. Molecules. 2021;26(10):2915. doi: 10.3390/molecules26102915.;.
15. Rahmawati, Astuti P, Wahyuono S. Review: Profil fitokimia dan multipotensi dari Coleus amboinicus (Lour.). Journal of Pharmacy and Science Clinical Research. 2021;6(2):158-88. doi: 10.20961/jpscr.v6i2.47436.;.
16. Arumugam G, Swamy MK, Sinniah UR. Plectranthus amboinicus (Lour.) Spreng: Botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016;21(4):369. doi: 10.3390/molecules21040369.;.
17. Taher M, El-Daly N, El-Khateeb AY, Hassan S, Elsherbiny EA. Chemical composition, antioxidant, antitumor and antifungal activities of methanolic extracts of Coleus blumei, Plectranthus amboinicus, and Salvia splendens (Lamiaceae). Journal of Agricultural Chemistry and Biotechnology. 2021;12(11):177-87. doi: 10.21608/jacb.2021.209208.;.
18. Cândido TM, Ariede MB, Pinto CAS de O, et al. Prospecting in vitro antioxidant and photoprotective properties of rosmarinic acid in a sunscreen system developed by QbD containing octyl p-methoxycinnamate and bemotrizinol. Cosmetics. 2022;9(2):29. doi: 10.3390/cosmetics9020029.;.
19. Sánchez-Campillo M, Gabaldon JA, Castillo J, et al. Rosmarinic acid, a photo-protective agent against UV and other ionizing radiations. Food and Chemical Toxicology. 2009;47(2):386-92. doi: 10.1016/j.fct.2008.11.026.;.
20. Cândido TM, Ariede MB, Lima FV, et al. Dietary supplements and the skin: Focus on photoprotection and antioxidant activity—A review. Nutrients. 2022;14(6):1248. doi: 10.3390/nu14061248.;.
21. Oliveira KB, Palú É, Weffort-Santos AM, Oliveira BH. Influence of rosmarinic acid and Salvia officinalis extracts on melanogenesis of B16f10 cells. Revista Brasileira de Farmacognosia (Brazilian Journal of Pharmacognosy). 2013;23(2):249-58. doi: 10.1590/S0102-695X2012005000135.;.
22. Satheesh V, Kaur J, Jarial S, et al. Indian borage: A comprehensive review on the nutritional profile and diverse pharmacological significance. The Pharma Innovation Journal. 2022;11(6):42-51.;.
23. Svobodová A, Psotová J, Walterová D. Natural phenolics in the prevention of UV-induced skin damage. A review. Biomedical Papers of the Medical Faculty of the University Palacký, Olomouc, Czech Republic. 2003;147(2):137-45. doi: 10.5507/bp.2003.019.;.

24. Saija A, Tomaino A, Trombetta D, et al. In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. International Journal of Pharmaceutics. 2000;199(1):39-47. doi: 10.1016/S0378-5173(00)00358-6.;.
25. Maruyama H, Kawakami F, Lwin TT, Imai M, Shamsa F. Biochemical characterization of ferulic acid and caffeic acid which effectively inhibit melanin synthesis via different mechanisms in B16 melanoma cells. Biological & Pharmaceutical Bulletin. 2018;41(5):806-10. doi: 10.1248/bpb.b17-00892.;.