



# The Effectiveness of Titanium Dioxide Sunscreen in Combination with Skin Defensil Plus® as an Anti-inflammatory Agent against Ultraviolet Radiation

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## Abstract

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**BACKGROUND:** Ultraviolet (UV) radiation can cause tanning and sunburn in the short-term; nevertheless, continued exposure to UV on unprotected skin will promote premature aging and gene mutation that leads to malignancy. Photoaging caused by sun exposure can happen through inflammation and reactive oxygen species pathway. Defensil plus® consists of natural plant ingredients that have an anti-inflammatory and anti-oxidant activity to protect the skin from UV exposure. Sunscreen with anti-oxidant and anti-inflammatory properties will reduce the unwanted effect of UV radiation, such as tanning and sunburn.

**AIM:** The aim of the study was to determine the effectiveness the combination of titanium dioxide (TiO<sub>2</sub>) sunscreen with defensil plus® against UV radiation.

**METHODS:** This was an experimental research design with pre- and post-test design approaches. Twenty subjects consisting of ten males and ten females with an average age of 23 years old, participated in this study. We conducted an experimental study with two groups. Group A was the TiO<sub>2</sub> + defensil plus® group; Group B was the TiO<sub>2</sub> group. The skin protection effect was compared by examining the erythema and melanin indexes using Mexameter® on day 0, post-radiation days 1, 2, 3, 4, and 7. Data Analysis using an independent t-test if the data distribution is expected, while the Mann-Whitney test is used if it does not meet the normality assumption. Data were considered significant with  $p < 0.05$ .

**RESULTS:** Melanin index decreased significantly from day 1 to 7 in the group that was given TiO<sub>2</sub> + defensil plus® compared to the group that was only given TiO<sub>2</sub> ( $p = 0.003$ ). The erythema index gradually decreased and significantly different in the group given TiO<sub>2</sub> + defensil plus® compared to the group given TiO<sub>2</sub> after 3<sup>rd</sup> day of application ( $p = 0.036$ ).

**CONCLUSION:** Application of sunscreen with TiO<sub>2</sub> + defensil plus® is effective in response to UV radiation, as demonstrated by lower erythema and melanin indexes than those treated with TiO<sub>2</sub> sunscreen alone, thus could protect the skin from UV radiation. Many studies showed that green and black tea (polyphenols) ameliorate adverse skin reactions following UV exposure.

## Introduction

The skin must be protected from sun exposure and maintain a balance of homeostasis. Exposure to ultraviolet (UV) light can cause sunburn, and pigmentation, long turn to premature aging, decrease the body's immune response to environmental pathogens, and increase the risk of premalignant and malignant neoplasms. At the molecular level, exposure to UV radiation can cause gene mutations that can lead to malignancy and immunosuppression [1], [2].

Sun protection is highly needed with the increasing incidence of melanoma, non-melanoma skin neoplasms, paraneoplastic disorders, and awareness of premature aging [3]. Sunscreen is one of the most important protections against UV rays; thus, wearing protective clothing and sunglasses cannot provide sufficient protection [4]. Titanium dioxide (TiO<sub>2</sub>) is one

of two UV filters the FDA categorized as generally recognized as safe and effective. This inorganic filter work by scattering, reflecting, and absorbing UV [5]. Studies have shown that herbs and herbal preparations could ameliorate adverse skin reactions following UV exposure [6]. Hence, we studied the benefit of defensil plus® consisting of *Ribes nigrum* (Black Currant) seed oil, *Cardiospermum halicacabum* Flower/Leaf/Vine Extract, *Rosmarinus officinalis* (Rosemary) Leaf Extract, and Tocopherol that have an anti-inflammatory and anti-oxidant activity to protect the skin from sun exposure in combination with TiO<sub>2</sub> thus can increase the effectivity of sunscreen [6], [7]. Photoaging can develop after prolonged UV radiation that activates reactive oxygen species and inflammatory pathways by activating the inflammatory transcription factor, the nuclear factor kappa beta (Nf-kB) [7], [8]. This study aims to compare the effectiveness of TiO<sub>2</sub> sunscreen ads with defensil plus® to TiO<sub>2</sub> sunscreen only on the erythema and melanin indexes.

## Methods

### Volunteers

The sunscreen was applied in the volar right or left forearm of 20 healthy volunteers (males and females in equal numbers), aged from 20 to 40 years (average 23 years old). All participants are of Indonesian nationality with skin phototypes III to IV.

The inclusion criteria of subjects in this research were: (1) Healthy male or female, aged 20–40 years, (2) willing to participate in the study and sign the informed consent, (3) have an even color on the volar forearm, do not have tattoos, skin spots, and scars wounds on the skin area to be studied, (4) not currently using photosensitizer drugs, (5) not currently taking corticosteroids, immunosuppressants, and NSAIDs both systemic and topical within 1 month before the time of the study, (6) not currently under skincare at the study site within 1 month before the study, and (7) not exposed to UV rays continuously within 1 month before the time of the study. The exclusion criteria were: (1) Pregnant or lactating women, (2) have a history of severe systemic disease, immune system disorders or malignancies, and nutritional deficiencies, and (3) have a history of irritation/allergy to cosmetics or sunlight (photosensitive). The ethical approval for this study was obtained from the Health Research Ethics Committee at the Dr. Moewardi General Hospital: 163/II/HREC/2021. All volunteers gave their written informed consent.

### Application of sunscreen

The investigated group of sunscreen was formulated by the same manufacturer. The products were divided into two groups: Group A consisted of TiO<sub>2</sub> with defensil plus, while Group B consisted of TiO<sub>2</sub> only.

After participants had acclimatized at room temperature for half an hour before the examination, each left/right volar forearm was marked two squares with 4 × 4 cm length. The group of sunscreens was spread to the rectangular areas as uninformedly as possible with the finger and allowed to remain on the skin for 15 min. Then, the erythema index and melanin index were measured using a mexameter (Khazaka MDD4®) for the baseline data (day 0).

### Data collections

The effectivity of sunscreen protection from UV radiation was examined on days 1, 2, 3, 4, and 7<sup>th</sup>. On each of the following days, the marked forearm, which had been applied a group of sunscreens for 15 min before, was exposed to artificial UVA and UVB (extrasolar glo®) light at 80 W at a 30 cm distance.

### Data analysis

The data were analyzed by parametric test using an independent t-test to determine if the data distribution was normal, previously tested for normality and homogeneity of data using the Shapiro–Wilk test.

The comparison of the mean skin protection effect against UV radiation on day 0 of baseline and day 0 of post-irradiation in each group was analyzed by paired t-test if the data distribution was normal. The Mann–Whitney and Wilcoxon tests are used if the data distribution is not normal. A statistical test is considered significant if  $p < 0.05$ . Research data analysis was calculated using SPSS for windows version 17.0 computer software.

## Results

A total of 20 volunteers completed the study and none presented any adverse reactions during the study.

The melanin erythema index between the two groups at the baseline shows insignificant differences; however, after exposure to UV radiation on day 1 and after, the defensil plus group significantly reduced than the non-defensil group (Table 1, Figure 1).

Our result showed that discontinuing using the two products on day 5 and day 6 increases the level of melanin index on day 7 in both groups.

The erythema index in group defensil plus was decreased and significantly different on days 3 and 4, with  $p = 0.036$  and  $p = 0.026$ , respectively. However, on day 7, the index slightly increased and made a insignificant difference with the non-defensil group (Table 2, Figure 2).

**Table 1: Comparison of the melanin index between Group 1 and Group 2**

Melanin index	Group A: TiO <sub>2</sub> +defensil plus® Mean ± SD	Group B: TiO <sub>2</sub> Mean ± SD	p-value
Day 0	169.39 ± 42.21	187.46 ± 30.31	0.062
Day 1	166.96 ± 37.76	189.36 ± 34.06	0.019*
Day 2	165.99 ± 36.33	184.97 ± 29.73	0.031*
Day 3	165.08 ± 34.28	189.73 ± 27.73	0.003*
Day 4	161.00 ± 39.77	186.71 ± 30.07	0.006*
Day 7	164.14 ± 46.03	187.96 ± 36.02	0.030*

\*Significant if  $p < 0.05$ . SD: Standard deviation.

## Discussion

The adaptive response of the skin to UV radiation exposure can be either pigmented or non-pigmented photoadaptation. Pigmentation is the result of melanocyte proliferation and an increase in photoprotective pigment. In contrast, non-pigmented photoadaptation is an adaptive response of the skin

**Table 2: Comparison of erythema index between Group A and Group B**

Erythema Index	Group A: TiO <sub>2</sub> +defensil plus® Mean ± SD	Group B: TiO <sub>2</sub> Mean ± SD	p-value
Day 0	220.70 ± 49.30	226.01 ± 38.71	0.644
Day 1	209.93 ± 45.12	233.77 ± 52.65	0.065
Day 2	209.68 ± 40.66	223.57 ± 48.06	0.232
Day 3	208.21 ± 37.79	230.50 ± 46.01	0.036*
Day 4	204.57 ± 40.71	230.09 ± 45.79	0.026*
Day 7	215.93 ± 40.84	220.41 ± 41.46	0.675

\*Significant if p < 0.05.

without pigment formation but with thickening of the stratum corneum or hyperkeratosis [9]. Pigmentation is an essential responsibility of the skin to UV radiation. Evaluation of pigmentation can be done using a simple Mexameter®, so the measurement of the melanin index is a good and ideal method to evaluate the effectiveness of sunscreens and skin whitening agents. The formation of melanin pigment or melanogenesis reaches its peak on day 4–7 after exposure to UV radiation [10].

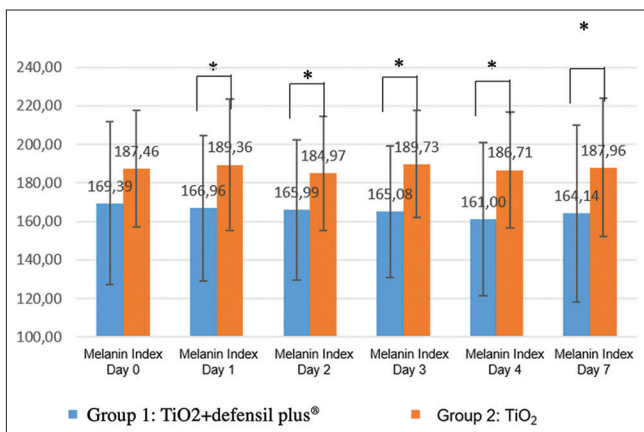


Figure 1: Melanin index diagram between Group A and Group B. \*Significant if p < 0.05

Our study reveals the superiority of defensil plus® to non-defensil plus® in reducing the melanin index and inhibiting skin pigmentation. This follows the study by Mudita *et al.* They reported that sunscreen could reduce the melanin index in the treatment group with healthy female subjects aged 20–45 years compared to the placebo group [9]. *In vitro* studies have also shown that TiO<sub>2</sub> can reduce the transmission of pigmentation [11]. Apriliani reported that sunscreens with TiO<sub>2</sub> and zinc oxide ingredients function to protect the skin from UV rays by scattering and binding keratinocytes due to UV radiation. Hence, there is a relationship between sunscreen use and the severity of melasma [12].

TiO<sub>2</sub> is a sunscreen with a broad spectrum that can protect the skin from UVA and UVB radiation. Chemically, TiO<sub>2</sub> is stable and does not react with other chemicals that can change the chemical composition or the surrounding environment. Natural chemicals, such as oils from plants and fruits, and antioxidants, have long-term beneficial effects, especially on skin damaged by free radicals from UV exposure [13]. *R. nigrum* (Black Currant) seed oil, *C. halicacabum* Flower/Leaf/Vine Extract, *R. officinalis* (Rosemary) Leaf Extract, and Tocopherol have anti-inflammatory effects

and antioxidant activity that can repair cell damage caused by UV exposure [14]. Tocopherol protects the skin from the negative effects of solar radiation by acting as a free radical scavenger. Several studies show that Vitamin E has anti-tumorigenic and photoprotective properties [15]. *Helianthus annuus* (Sunflower) is a natural ingredient for skin protection as a topical sunscreen containing the active substance quercetin, which is anti-inflammatory and antioxidant and repairs cell protein damage [6]. Research conducted by Yamada *et al.* reported that the aqueous extract of *R. officinalis* (Rosemary) has antioxidant activity and inhibits melanin production, which clinically demonstrated that this extract could remove browning and sagging of the skin and restore skin firmness. In addition, the ethanolic extract of *R. officinalis* (Rosemary) also has the effect of antioxidant activity, inhibition of melanin production, and inhibition of cancer cell proliferation. The melanin and keratinocyte are the potentials to be blocked by antioxidant and anti-inflammatory agents on defensil plus®, and the proinflammatory cytokines will be blocked. Thus, using defensil plus® since the 1<sup>st</sup> day of UV radiation will lower the melanin index [16].

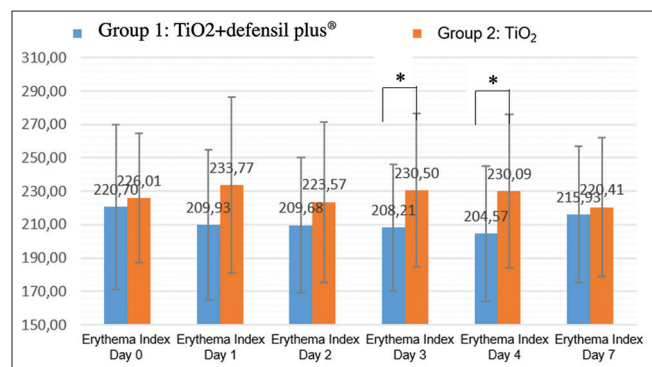


Figure 2: Erythema Index between Group A and Group B. \*Significant if p < 0.05

UV radiation can cause sunburn, which is characterized by an acute inflammatory response that can be characterized by erythema, edema, burning sensation, itching, and pain in the exposed area [17] Erythema is the primary response of the skin after exposure to UV radiation which is visible to the naked eye and can be measured objectively using a Mexameter®. The inflammatory response in the form of erythema on the skin after UV exposure reached its peak on the 2<sup>nd</sup> day. Measurement of erythema index with Mexameter® is a simple and straightforward procedure, so it is ideal to evaluate the effectiveness of sunscreen products in preventing erythema after exposure to UV light [10].

The erythema index was gradually lower in the defensil plus® group compared to the non-defensil plus® group. This is due to defensil plus®'s anti-antioxidant and anti-inflammatory effects that diminish the size of the blood vessel and flushing. The combination effect of TiO<sub>2</sub> and defensil plus® is synergistic and non-irritating. Sunscreen creams can



absorb or reflect UV radiation and protect the skin from sunburn. TiO<sub>2</sub> is the market's most widely used sunscreen agent and is known to protect the skin from inflammation and pain from sunburn [18]. TiO<sub>2</sub> has been used as a physical sunscreen for many years due to its ability to strongly absorb UV rays that can induce radiation-induced erythema of the skin. Not only effective against UV radiation, but TiO<sub>2</sub> also protects against infrared radiation and visible light [19].

TiO<sub>2</sub> and defensil plus® give the effect of decreasing the erythema index level. The ideal sunscreen is inert and non-irritating, has a photostability effect, and can be mixed with other ingredients [20]. Unlike other organic sunscreens, TiO<sub>2</sub> sunscreens are inert, making them the least likely to cause skin irritation and sensitization. Due to its chemical inertness and lack of photoreactivity, TiO<sub>2</sub> is the sunscreen of choice for individuals with sunscreen allergies and children [19]. Defensil plus® contains *R. nigrum* (Black Currant) seed oil, *C. halicacabum* Flower/Leaf/Vine Extract, *R. officinalis* (Rosemary) Leaf Extract, *H. annuus* (Sunflower) and Tocopherol which is a natural chemical that has anti-inflammatory properties that can prevent sunburn, manifests as skin erythema due to UV exposure [21].

Research conducted by Ashigai *et al.* shows that *R. nigrum* (Black Currant) reduces skin dehydration caused by UV radiation. In addition, there were a decrease in the transcriptional levels of interleukin (IL) 6 and matrix metalloproteinases in mouse skin, indicating that *R. nigrum* (Black Currant) increased skin hydration in UV-irradiated mice [22]. Research conducted by Tsai *et al.* showed that *R. officinalis* (Rosemary) contributes to anti-inflammatory properties for cosmetic or dermatological products. Although the mechanism underlying the effects of Rosemary extract is not known in detail, it may involve inhibition of NF-κB activation and TLR2 expression [23].

Research conducted by Tamara *et al.* regarding the determination of the potency of sunscreen based on sunflower seed oil (*H. annuus*) in cream preparations with a combination of oxybenzone and octyl methoxycinnamate *in vitro* showed that the SPF value was statistically significant, so it can be concluded that the formula with the addition of sunflower seed oil was substantial in inhibiting erythema due to UV exposure [24]. Trevithick *et al.* reported that erythema, edema, and skin sensitivity commonly associated with UVB-induced reduction were significantly reduced with topical application of tocopherol even after exposure. In addition, these observations suggest that sunburn therapy can be performed even after irradiation is discontinued, with d-alpha-tocopherol derivatives, which are stable against autoxidation [25].

## Conclusion

The application of SPF 30 TiO<sub>2</sub> sunscreen combination with defensil plus® is more effective against the response of UV radiation, indicated by the lower index of erythema and melanin measurement than that of non-defensil plus sunscreen.

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