



## Ethanol Extract Cream of Jackfruit Tree Bark (*Artocarpus Heterophilus*) is Equally Effective with Hydroquinone Cream on Preventing the Increase of Melanin on Guinea Pigs Skin (*Cavia porcelus*) Exposed to Ultraviolet B Ray

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### Abstract

**Introduction:** Ethanol extract of jackfruit tree bark (*Artocarpus heterophyllus*) contains antioxidants, phenolic substances, *tannin*, steroid, *linoleic acid ethyl ester*, and vitamin C which can inhibit the elevation of melanin levels on epidermal tissue. Hydroquinone (HQ) was used as comparison because HQ is the gold standard of hyperpigmentation therapy. **Method:** This research is a laboratory experimental research using randomized post-test only control group design with 30 male guinea pigs (*Cavia Porcelus*) divided into 3 groups with 10 individuals on each group, with control group given base cream, group 1 was administered 4% hydroquinone cream, and group 2 was administered 4% jackfruit tree bark extract cream. All intervention groups were exposed with UV-B ray with total dose of 390 mJ/cm<sup>2</sup> three times in a week for 2 weeks, and underwent biopsy of their skin sample to examine the amount of melanin on the epidermal layer. **Result:** The result of our research showed the mean amount of melanin on control group was 54.33±4.51%, group 1 was 3.01± 0.89% and group 2 was 4.23± 1.82%. There is a significant difference between control group and both group 1 and 2 in inhibiting the increase of melanin amount on epidermal tissue (p<0.05). There is no significant difference between group 1 and 2 on inhibiting the increase of melanin amount on epidermal tissue (p>0.05). **Conclusion:** We conclude that 4% jackfruit tree bark extract cream can prevent the increase of melanin amount on guinea pigs' skin epidermal layer. The 4% jackfruit tree bark extract cream is equally effective to 4% hydroquinone cream on preventing the elevation of melanin amount on epidermal layer of guinea pig's skin exposed with UV-B ray.

**Keywords:** Jackfruit tree bark extract cream, Melanin amount, UV-B ray.

### Introduction

Aging is a physiologic process that will be experienced by every people, but the effect incited by the aging is the one that must be treated or even prevented. Factors precipitating aging are external factors such as sunray, air pollution, cigarette smoke, and medicines, while internal factors are genetics, race, hormonal, Reactive Oxygen Species (ROS), and free radicals [1]. A disease caused by ultraviolet ray is called photo aging. ROS can stimulate the inhibition of the work of tyrosinase enzyme,

directly disrupting melanocyte cells, hastening the degradation of melanosome, and inhibiting the synthesis of melanogenesis enzyme, preventing the elevation of melanin amount caused by UV-B exposure [1, 2]. Jackfruit tree bark extract (*Artocarpus Heterophyllus*) contains phenol substance, antioxidant, vitamin C, tannin, steroid, and linoleic acid ethyl ester, which has antioxidant effect and photoprotective effect against melanogenesis, which causes darkening effect on the skin, which in turn

can cause psychosocial disturbance. Ultraviolet exposure can cause formation of ROS, which can stimulate the formation of free radicals and stimulating melanogenesis process, increasing melanin amount. Hydroquinone is the gold standard for therapy of melasma. Hydroquinone's mechanism of action is through degradation of tyrosinase, causing inhibition of melanogenesis, resulting in the prevention of melanin amount elevation and protecting skin from free radicals. A research conducted by Hastiningsih et al. Concluded that 4% concentration of jackfruit tree bark extract cream is capable of inhibiting the elevation of melanin amount [2]. Therefore, investigation jackfruit tree bark extract was chosen to be proved in this research .

## Material Methods

### Study Design

This research is an experimental research using posttest only control group design conducted *in vivo*; using 30 male guinea pigs (*Cavia Porcelus*) aged 3 months weighing from 300-350 grams as experimental animal, divided into 3 groups' randomly containing 10 guinea pigs each. The three groups were control group (negative control), given base cream (without hydroquinone or jackfruit tree bark extract creams); group 1 (positive control), given 4% hydroquinone cream; and group 2 (intervention group), given 4% jackfruit tree bark extract cream. All groups were given cream and exposed with UV-B ray.

### Equipment

Equipment used in this research are guinea pigs kennel, drinking bottles, UV-B lamps (fluorescent lamp PL-S 9 W/01/2P Medical with gel-nb-UVB-311 nm wave, Philips), shaving machine Goal, Tanita digital weight measurement tool, surgical equipment such as anatomical scissors, scalpel, equipment for preparation of histological slides such as microtome, object glass, and covering glass, Olympus CX41 microscope (*Olympus, Japan*), *Optilab Pro* camera (*Micronos, Indonesia*), and a ruler.

### Materials

Male guinea pigs, local strain, aged 3 months weighing from 300-350 grams, Masson-Fontana reagents, 4% jackfruit tree bark extract cream (*Arthocarpus Heterophyllus*)

4% hydroquinone cream, and base cream (not containing active substances of 4% hydroquinone or 4% jackfruit tree bark extract).

## Research Procedure

The jackfruit tree barks was made into ethanol extract using 96% ethanol solution, and subsequently were made into 4% extract cream. The creams were applied to the designated group, and all of the guinea pigs were exposed with UV-B ray 3 times a week with the dose of 65 mJ/cm<sup>2</sup> as long as 130 seconds in each session, with total dose of UV-B received for 2 weeks is 390 mJ/cm<sup>2</sup> until the end of the application of the 4% jackfruit tree bark extract and 4% hydroquinone creams.

The biopsy sample was taken from the epidermal layer of the guinea pigs' skin, and was made into histological slides. The examination of the amount of melanin of the guinea pigs skin epidermal layer was conducted using *Optilab Pro* camera and *Olympus CX41* microscope with 400x magnification using *Masson-Fontana* dye.

## Data Analysis

Analysis in current study using SPSS version 17.0 for windows. Descriptive analysis was conducted as a basis for statistical analysis. Comparative analysis was conducted using One Way ANOVA test, because the data were normally distributed and homogenous, and subsequently, to determine difference between each group, least significant difference (LSD)/Post Hoc test was conducted. All value considered significant if  $p < 0.05$ .

## Results

### Descriptive Analysis

The result of descriptive test on the mean amount of melanin on each group were presented in Table 1, can be seen from the descriptive analysis about the mean amount of melanin that on the control group that was given base cream and UV-B exposure, the amount of melanin is higher than group 1 (UV-B and 4% hydroquinone cream) and group 2 (UV-B and 4% jackfruit tree bark extract cream), as much as  $54.33 \pm 4.51\%$ , while the mean amount of melanin in group 1 is higher than group 2, that is  $3.01 \pm 0.89\%$ , compared to  $4.23 \pm 1.81\%$  of group 2.

**Table 1: Descriptive Analysis of Melanin's Mean Amount of Each Group**

Melanin	n	Mean (%)	Std Deviation
UV-B ray and base cream	10	54.33	4.51
UV-B and 4% hydroquinone cream	10	3.01	0.89
UV-B and 4% jackfruit tree bark extract cream	10	4.23	1.81
Total	30	20.52	24.47

### Normality and Homogeneity Test

The data of melanin amount were tested of its normality using Shapiro-Wilk test. The

result showed that data were normally distributed ( $p > 0.05$ ) and have a homogenous variant ( $p > 0.05$ ) (Table 2).

**Table 2: Normality and Homogeneity Test on Melanin**

Subject Groups	n	p
<b>Normality test</b>		
Melanin amount of control group	10	0.963
Melanin of group 1		0.219
Melanin of group 2		0.553
<b>Homogeneity test</b>		
Melanin	F	p
	2.52	0.053

### Comparison of Melanin between Groups Intervention

The effect of the intervention was analyzed based on the amount of melanin between each group after intervention of UV-B exposure and application of jackfruit tree bark extract cream. Table 4, showed that the mean amount of melanin on control group is

$54.33 \pm 4.52$ , the mean amount of melanin on group 1 is  $3.01 \pm 0.89$ , and the mean amount of group 2 intervention is  $4.23 \pm 1.82$ . Significance analysis using One Way ANOVA test showed the value of  $F = 1050.00$  and  $p = 0.001$ . This means that the amount of melanin on the three groups after intervention were significantly different ( $p < 0.05$ ).

**Table 4: Difference of Melanin Amount Between Groups After Administration of UV-B Ray and Jackfruit Tree Bark Extract**

Study Group	n	Melanin (mean $\pm$ SD) (%)	F	p
Control group	10	$54.33 \pm 4.52$	1050.00	0.001
Group 1		$3.01 \pm 0.89$		
Group 2		$4.23 \pm 1.82$		

**Table 5: Post hoc comparison of Melanin Amount Between Control Group and Intervention Groups**

Groups	Mean differences	p
Control Vs. Group 1	51.32	0.001
Control Vs Group 2	50.10	0.001
Group 1 Vs. Group 2	1.22	0.349

There was a significant difference in the amount of melanin between control group and group 1 and group 2 ( $p < 0.05$ ) and there was no significant differences in the amount of melanin between group 1 and group 2 ( $p > 0.05$ ), it means there was no differences effect between hydroquinone group with jack fruit tree bark cream in reducing melanin (Table 5).

Control group, exposed with UV-B and being applied base cream showed dark brown/hyperpigmentation on the skin (Figure 1A). Group 1, exposed with UV-B and being applied with 4% hydroquinone cream, showed the guinea pigs' white skin (Figure 1B). Group 2, exposed with UV-B and being applied with 4% jackfruit tree bark extract cream showed white colored skin with subtle

dark streak stains (Figure 1C). Histological skin examination reveals control group (negative control), given UV-B and base cream, showed brown/black colored dense melanin on guinea pig's epidermal tissue, white arrow showed the density of melanin (Figure 2A).

Group 1 (positive control), given UV-B and 4% hydroquinone cream, showed much less dense pink colored melanin on epidermal tissue, black arrow showed the very decreased melanin (Figure 2B). Group 2, given UV-B and 4% jackfruit tree bark extract cream, showed less dense light purple colored melanin on epidermal tissue, black arrow showed the decreased melanin density (Figure 2C).



Figure 1: A, 1B, 1C skin appearance between group after exposure of UV-B rays and topical treatment (1A control group; 1B melanin group; 1C Jack fruit tree bark extract)

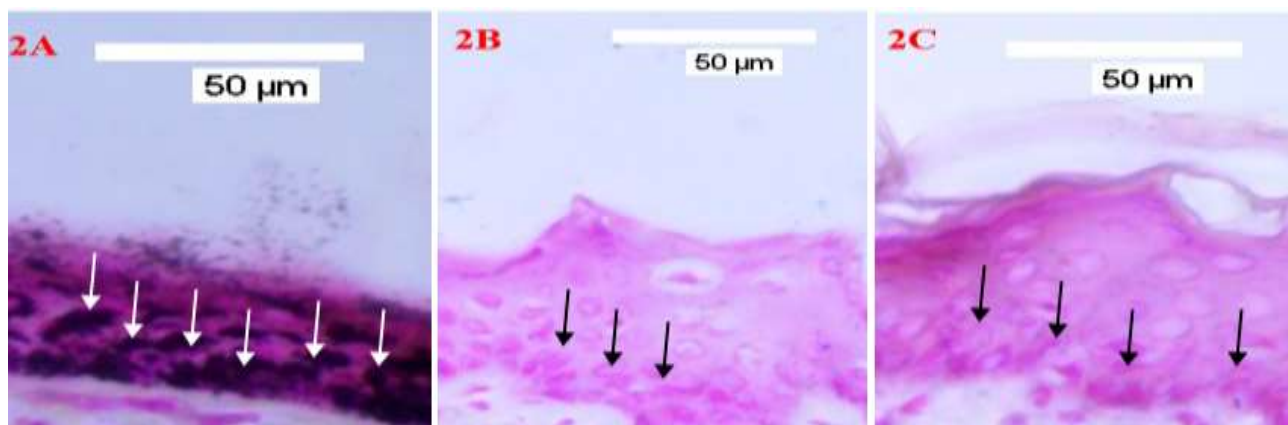


Figure 2: A, 2B, 2C histological skin appearance between group after exposure of UV-B rays and topical treatment (2A control group; 2B melanin group; 2C Jackfruit tree bark extract)

## Discussion

### Effects of UV-B on Melanin

Exposure of UV-B rays on skin can reduce endogenous antioxidant effect on every layer of skin such as *glutathione* (GSH), *Superoxide dismutase* (SOD), *catalase*, and *ubiquinol*, and also produce free radicals such as *Hydrogen Peroxidase*, *Anion Superoxide*, *Nitric Oxide*, resulting in production of *reactive oxygen species* (ROS) [3, 4]. Skin damages caused by sunray exposure are parallel to frequency and length of the exposure, type of UV rays, and the amount of melanin on skin (one's skin type). Clinical symptoms caused by this photoaging process are skin wrinkles and hyperpigmentation [3].

UV radiation caused pigmentation through several ways, by enhancing the work of melanogenic enzyme, through DNA disruption that stimulates melanogenesis, increasing melanosome transfer to keratinocyte and increases dendritic activity of melanocyte cells [5]. UV radiation can stimulate formation of ROS, which in turn stimulates the release of *Nitrite Oxide* (NO), Protein Kinase, Melanocyte Stimulating Hormone (MSH), and PGE<sub>2</sub>, which can

stimulate melanogenesis process, which stimulates the formation of melanin by melanocyte [6].

### Effects of Hydroquinone Cream on Melanin

Hydroquinone (HQ) is the gold standard for the therapy of hyperpigmentation/ melasma and has the mechanism of work by inhibiting tyrosinase enzyme, directly damaging melanocyte, hastening melanosome degradation, and inhibiting melanogenetic enzyme [1]. HQ can reduce hyperpigmentation lesions as much as 90%, capable of preventing the increase of melanin amount on epidermal layer [1, 7, 8].

### The Effects of Jackfruit Tree Bark Extract Topical Cream on Melanin

Jackfruit tree bark extract (*Artocarpus Heterophyllus*) taken from Sibang village, Abiansemal district, Badung region, Bali has undergone phytochemical analysis by Hastiningsih et al. And from the extract was found antioxidant substances (987.42 Ppm GAEAC), phenol (0.49 % b/b GAE), tannin (0,86 % b/b TAE), and Vitamin C (31.94 mg/100g). This extract also underwent Gas Chromatography-Mass Spectrophotometry

(GC-MS) analysis, and was found to contain Hexadecanoic acid ethyl ester, Estra-1,3,5(10)-trien-17-beta-ol, Ethyl tridecanoate, Linoleic acid ethyl ester, Ethyl Oleate, and Gamma Sitosterol. Based on the phytochemical analysis and GC-MS, jackfruit tree bark extract has antioxidant effects capable on reducing negative effects from oxidants, including enzymes and metal binding proteins. Reduction effects from antioxidants were done through 2 ways, 1) preventing the excess deposition of oxidant substances, and 2) preventing continuous chain reaction [9]. Antioxidants can bind free radicals. Botanical antioxidants were proved to be able to reduce the events of photocarcinogenesis and photoaging caused by ROS elevation [10].

Hexadecanoic acid ethyl esters, Ethyl tridecanoate, are also antioxidants [2]. Antioxidants prevent ROS formation, which can stimulate melanogenesis process. By the inhibition of melanogenesis process, the elevation of melanin amount caused by UV-B ray is prevented. Polyphenols have also protective effects against UV radiation on skin, preventing skin damages and cancers. Polyphenols have anti-inflammation effects; an immunomodulator effect, able to repair damaged DNA, capable of repairing cell functions, and has a photo protective role [11, 12]. Because of that, the presences of polyphenols are able to inhibit melanogenesis process, preventing the elevation of melanin amount. Tannin has antioxidant characteristics and has the ability as an anti tyrosinase [13].

Because of the inhibition of melanin's biosynthesis process, the elevation of melanin production after UV-B exposure is not manifested. Vitamin C is named as antioxidants because its ability to donate its electrons. When vitamins C donate one of its electrons, vitamin C turned into free radicals (semi-dehydroascorbic acid or radical ascorbic. Compared to other free radicals, radical ascorbic is more stable and unreactive. The reductions of reactivity of free radicals into unreactive free radicals are called free radicals scavenging or sequencing (binding). Because of that, vitamin C is a

good free radical binder [11]. Because of the effects of vitamin C, ROS aren't formed and melanogenesis processes can be inhibited, preventing the increase of melanin amount. Estra-1, 3, 5(10)-trien-17-beta-ol, Gamma Sitosterol is one of many members of steroid group. The mechanism of reduction of melanin amount by steroids is through oxidation of tyrosinase enzyme enzymatically into cytotoxic products in melanocytes, causing degeneration/ disruption of pigment cells, causing depigmentation [14]. Linoleic acid ethyl ester, is capable of degrading tyrosinase, inhibiting biosynthesis of melanin, and subsequently, inhibiting the elevation of melanin caused by UV-B exposure. Addition of antioxidant substances into creams are increasing. Topical antioxidants are useful to suppress ROS effects on skin. The base cream of oil inside water become the main choice of topical antioxidants because of its stability, easy to absorb, and easy to wipe [15].

Besides of that, application of antioxidants inside creams can increase skin humidity and reduces trans-epidermal water loss (TEWL) [16, 17]. Jackfruit tree bark extract on this research has no norartocarpetin and artocarpesin contained because of our lack of equipment to extract them. Norartocarpetin and Artocarpesin based on previous research are flavonoids having the effects as competitive enzyme tyrosinase inhibitory, but in this research, it is proven that the jackfruit tree extract can prevent the elevation of melanin amount on guinea pigs' skin exposed with UV-B. This is due to the extract has several substances with antioxidant effects, photoprotective, and tyrosinase- degrading, causing potentiation effects on the prevention of melanin elevation.

## Conclusion

Administration of jackfruit tree bark extract cream (*Artocarpus Heterophyllus*) 4% prevents the elevation of melanin amount on guinea pigs (*Cavia Porcelus*) exposed with UV-B ray and has the equal effectivity on preventing the increase of melanin amount on guinea pigs' skin exposed with UV-B ray.

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