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Effectiveness of *Artocarpus heterophyllus* Lamk. serum in eliminating dark spot in the guinea pig melasma model due to UV-A exposure

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ABSTRACT: Indonesia's high UV-A exposure significantly contributes to skin hyperpigmentation conditions such as melasma. This study investigated the effectiveness of Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum in reducing dark spots in a guinea pig model of melasma induced by UV-A radiation. Crude extracts were prepared and purified from Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves, then formulated into serums at 4% and 5% concentrations. These formulations underwent physical quality evaluations, including organoleptic properties, pH, homogeneity, spread ability, adhesion, and viscosity. Anti-hyperpigmentation effects were assessed using Masson Fontana staining on UV-A-exposed guinea pigs treated for 14 days. The results demonstrated a reduction in melanin content, with mean values of 17.97±1.68 (negative control), 1.17±0.28 (positive control), 3.56±2.77 (4% serum), and 2.56±1.25 (5% serum). Histological analysis showed that fibroblast proliferation and epidermal hyperplasia were significantly reduced with serum treatment. These effects are attributed to the flavonoid content in Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves, which exhibit antioxidant and anti-melanogenesis properties. In conclusion, Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum, particularly at 5% concentration, is effective in reducing melasma-related hyperpigmentation and may offer a safer alternative to synthetic treatments.

KEYWORDS: *Artocarpus heterophyllus* Lamk; Melasma; Serum; UVA rays.

▪ INTRODUCTION

Indonesia is a country located on the equator, has a tropical climate with high exposure to sunlight [1]. UV rays are part of the sunlight spectrum, consisting of UVA, UVB and UVC. Exposure to UV light has beneficial effects such as the formation of vitamin D and killing bacteria, but also bad effects. UV radiation penetrates the skin and is absorbed by chromophores, molecules that initiate photobiological reactions [2]. UVA rays, which reach 95% of the earth's surface, have low energy but excessive exposure can cause sunburn, photo aging, tanning, and skin hyperpigmentation known as melasma [3].

Melasma, or chloasma, is characterized by darkened patches that commonly develop on the face, such as the upper lip, cheeks, and chin, and may extend to the neck. These spots are typically light brown, dark brown, or black and have an irregular shape [4]. Women have a 9 times higher risk of developing melasma than men, especially at the age of 20-50 years and during pregnancy. Melasma treatment begins with preventing risk factors, protecting from UV rays, and reducing skin damage by minimizing side effects. Typical topical treatments include hydroquinone (HQ), tretinoin, salicylic acid, acid, kojic acid, glycolic acid, kojic acid. While HQ is considered the gold standard, improper dosage or prolonged use can lead to severe side effects, including the risk of cancer [5].

The use of herbal ingredients shows promise as an effective approach for treating dark spot melasma and has relatively few side effects compared to synthetic cosmetics. Plants such as Jackfruit (*Artocarpus heterophyllus* Lamk.) contain flavonoid compounds which can inhibit the melanogenesis process by reducing the activity of tyrosinase and DOPA oxidase, as well as inhibiting the spread of melanin in the epidermis layer

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[6]. Jackfruit (*Artocarpus heterophyllus* Lamk.) is also rich in polyphenols and antioxidants which can protect the skin from UV rays [7]. Serum is an effective choice of preparation for treating melasma because it has a high concentration, can penetrate the skin more deeply, has a low viscosity, and forms a thin film on the skin surface [8].

This study aimed to evaluate the effectiveness of Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum at 4% and 5% concentrations in treating UV-A-induced melasma in a guinea pig model. The objective is to assess both the serum's physical characteristics and its biological effects, particularly its ability to reduce melanin content, epidermal hyperplasia, and promote fibroblast proliferation, thereby supporting its potential as a natural and safer alternative to conventional melasma treatments.

▪ MATERIALS AND METHODS

Materials

Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves, 70%, 95% and 100% ethanol, filter paper, technical ethyl acetate, FeCl3, technical acetic acid, mayer, dragendorff, sulfuric acid, hydrochloric acid, hydrogen, distilled water, mg powder, anhydrous acetic acid, aluminum foil, chloroform, sodium benzoate, glycerin, potassium sorbate, xanthan gum, buffered formalin, silver nitrate fontana, dH2O, gold chloride 1%, sodium thiosulfate 5%, nuclear fast red, xylene (Indonesia).

Intruments

Blender (Philips), sieve (ABM), analytical balance (Ohaus), digital scale (ACIS), glassware (Pyrex), separating funnel (Iwaki), evaporator (Buchi), oven (Memmert), furnace (Thermolyne), desiccator (Shuniu), water bath (Memmert), syringe (One med), razor blade (Gols), homogenizer (HR-500DG), magnetic stirrer (Thermo), crucible, Brookfield viscometer (US SOLID), pH meter (Starter 5000), mortar (One med), stamper (One med), UV A lamp (Exo Terra Heat Lamp), vacuum pump filter, UV meter (UV Light Meter).

Methods

Simplisia preparation

The preparation of simplisia begins by selecting Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves that are at an optimal maturity—not too young and not too old—as this stage contains the highest levels of metabolite compounds [9]. Once collected, the leaves are cleaned and dried under direct sunlight for five days. During the drying process, the leaves are placed on a clean surface and covered with a black cloth to protect them from contaminants and excessive UV exposure.

Extract preparation and purification of jackfruit (Artocarpus heterophyllus Lamk.) leaf

Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves were pulverized into simplisia powder, then put into a glass jar and 70% ethanol was added in a ratio of 1:4. The jar is then securely sealed and covered with black plastic. Stir the mixture occasionally, and after 24 hours, strain it using filter paper. The filtration results were stored in a bottle, and the macerate was re-extracted with additional solvent, this process was repeated up to 3 times. The resulting Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract was used for the purification process. Twenty grams of 70% ethanol extract were added to an Erlenmeyer flask, mixed with 400 mL of hot water, and stirred until fully dissolved. The suspension was put into a separatory funnel and 400 mL of ethyl acetate was added in a 1:1 ratio. The mixture was shaken for one minute before standing. Two layers were formed: a 0.8 g/mL ethyl acetate layer on top, and a 1 g/mL water layer below. Ethyl acetate fractionation was carried out in two stages to obtain a clear fraction. The fraction was then solidified using a rotary evaporator [10].

Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract standardization test

Specific parameters

The percentage of water-soluble compounds was evaluated by distilled five grams of extract with 100 parts water and chloroform for 24 hours. The mixture was shaken for 6 hours, then left for 18 hours before filtering.

Twenty milliliters of the filtrate were evaporated, and the residue was heated at 105°C until a constant weight was obtained, determining the percentage of water-soluble compounds based on the extract's initial weight [11].

The percentage of ethanol-soluble compounds was evaluated by macerating five grams of extract were soaked in 100 ml of ethanol 95% distilled water for 24 hours, heated for 6 hours, then 18 hours more. After sealing, 20 ml of filtrate was evaporated, and the residue was heated at 105°C until stable. The percentage was based on the initial extract weight [11].

Non-specific Parameters

The loss on drying of extract were measured by weighing one gram of extract, spread in a preheated crucible, and dried at 105°C until the weight stabilizes. After cooling, the final weight is used to calculate the percentage of weight loss [11].

The acid-insoluble ash content is determined by boiling 25 ml of dilute hydrochloric acid R for 5 minutes. The undissolved residue is filtered, washed with hot water, and weighed. The percentage is calculated based on the sample's initial weight [11].

Preparation of Jackfruit (*Artocarpus heterophyllus Lamk.)* leaf serum

The preparation of serum was described on Table 1. Xanthan Gum is added 20 times water in a beaker glass, then stir until an emulsion corpus is formed, then add glycerin little by little while continuing to stir ad homogeneous, grind potassium sorbate in a mortar then add sodium sorbate and Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract stir until homogeneous, then add aquadest ad 60mL stir until homogeneous and store in a container [12].

Table 1. Serum formulation of *Artocarpus heterophyllus* Lamk.

Material	Weight
Jackfruit (*Artocarpus heterophyllus* Lamk.) Leaf Fraction	4% and 5%
Xanthan Gum	0,5
Glycerin	10
Potassium Sorbate	0,1
Sodium Benzoate	0,1
Aquadest	Ad 100

Physical quality test of jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum

The physical quality test was conducted by evaluating the organoleptic test, pH, homogeneity, spread ability, stickiness, and viscosity. The organoleptic test was conducted by observing changes in the shape, color, and odor of the serum. The pH was measured using a pH meter inserted into the serum preparation. For cosmetic preparations to be suitable for the skin, the pH level should match the natural pH range, which is 4.5 to 6.5 [13]. The homogeneity was evaluated by placing the serum on the glass object evenly and the sample should show homogeneous results and be free of clumpy particles [13]. The spread-ability was done by weighing a sample approximately 0.5 g is placed on the caul and left for one minute. Subsequently, 150 g of load was added, and after 1 minute, the diameter was measured again to ensure it remained [14]. The stickiness was evaluating by placing a 0.25 g sample between two glass plates on the adhesion test device. A 1 kg weight was applied for 5 minutes, after which the weight was removed and replaced with an 80 g load. The serum release time was then recorded [14]. The viscosity of the serum was determined using a Brookfield Viscometer (Ametek, DV2T, USA). The serum is placed in a container and turns into a crust around it. The speed and spindle number were adjusted so that the instrument's needle could read a scale from 0 to 100 [15].

Total flavonoid assay

An 80 mg sample of the extract was weighed, dissolved in a small amount of ethanol, and placed into a 10 ml volumetric flask. It was then homogenized using a sonication device for 30 minutes before the extract solution was filtered. Serum samples were measured sample solution equivalent to 100mg of extract, dissolved with a little ethanol and put into a 50 mL volumetric flask and then homogenized. Each as follows: 0.5 mL of sample solution, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1N sodium acetate, and 2.88 mL of distilled water. The materials were then added into a test tube and mixed well. The mixture was incubated for thirty minutes, after which it was dried and the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer (Shimadzu UV-1900i (Japan).

Antioxidant activity 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method

The prepared sample with 100 mg extract was mixed with a few milliliters of ethanol and then poured into a 50 mL flask. The volume was set to the mark, and the solution was mixed thoroughly. Each 4% serum solution was pipetted with a concentration of 0.200; 0.250; 0.300; 0.350; 0.400, entered into the measuring 5 ml. Then add 1 mL of 0.4 mM DPPH solution into the volumetric flask and measure the volume to the limit mark with ethanol and homogenized obtained 4% test solution with a concentration of 80; 100; 120; 140 and; 160µg/ml 5% serum solution was pipetted respectively 0.025; 0.050; 0.075; 0.100; 0.125, inserted into a 5 mL volumetric flask. Next, 1 mL of soluble DPPH (0.4 nM) was added to the volumetric flask and stirred to the mark with ethanol p.a. This resulted in 5% homogeneity with concentrations of 10, 20, 30, 40, and 50 µg/mL. Each subject was bathed for thirty minutes in a dark environment in the room. The samples were then measured using a UV-Vis (Shimadzu UV-1900i (Japan) at 516 nm.

Antihyperpigmentation effectiveness test

The anti-hyperpigmentation effectiveness test was conducted in accordance with Ethical Clearence number DP.04.02/F.XXXII.25/0713/2024. Guinea pigs were adapted for 7 days, then their back hair was shaved about 3 cm and irradiated with UVA at a dose of 100 J/cm² for 5 minutes every day for 6 days. Subsequently, the guinea pigs were randomly assigned to 4 groups, with each group containing 4 animals. Group 1 served as the negative control (using serum base material), Group 2 as positive control using hydroquinone serum (Cos De Baha, South Korea), Group 3 was given 4% Jackfruit *(Artocarpus heterophyllus* Lamk.) leaf serum, and Group 4 was given 5% Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum. The serum was applied once a day for 14 days. After 14 days of treatment, guinea pig skin and tissues were taken for further analysis. Anti-hyperpigmentation effectiveness testing was conducted using the Masson Fontana method, while epidermal thickening was tested using the Hematoxylin- Eosin staining method [15].

▪ RESULTS & DISCUSSION

Extract preparation and purification of jackfruit (*Artocarpus heterophyllus* Lamk.) leaf

Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves that have been refined are then weighed to determine their weight [16]. Additionally, the leaves are sun-dried by covering them with a black cloth and placing them on a base to prevent contamination from foreign objects. In addition, it is covered with a black cloth to prevent direct UV light until the appropriate level of fragility [17]. The dried leaves of *Artocarpus heterophyllus* Lamk. were then pulverized using a blender and sieved using 40 mesh sieving, sieving with a mesh size of 40 was used because it could produce the optimal amount of extract [18]. In making Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract, the maceration method employs a 70% ethanol solvent. This straightforward technique is used for plant materials that are heat sensitive [19]. The reason for choosing 70% ethanol as a solvent is because of its ability to attract active compounds more effectively than other organic solvents. Ethanol has a boiling point of only 79°C, indicating that it requires less heat energy for the concentration process. In addition, ethanol is the only solvent that is safe and non-toxic if consumed, because the level of toxicity is lower than other solvents. Another reason for choosing 70% ethanol as a solvent is because flavonoid compounds are usually in the form of polar glycosides, so they require solvents that are also polar to dissolve. Ethanol 70% is one of the polar solvents suitable for this purpose [20]. Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves were soaked with 70% ethanol for 1x24 hours and covered with aluminum foil. Maceration was performed three times to

ensure the maximum extraction of chemical compounds from the leaf sample. During soaking, the simplisia is kept in a sealed container and shielded from direct light to prevent light-induced reactions or color changes [21]. Additionally, the macerate is filtered under vacuum to obtain the desired filtrate. Next, the filtered results were filtered using a rotary evaporator to produce concentrated extracts and the amount filtered out. The result showed a yield of 11%.

The concept of "like dissolve like" explains that compounds dissolve in solvents with comparable chemical properties. The 70% ethanol extract was suspended in more polar hot water, which was effective for extracting flavonoid compounds that are generally polar. This process was followed by purification using ethyl acetate to remove sugars and other nonpolar fractions, while n-hexane was used to dissolve nonpolar compounds such as fats and terpenoids. Phytochemical screening showed positive fractions containing flavonoids, tannins, and steroids. This approach uses hot water and ethyl acetate to increase flavonoid levels which are purer than n-hexane which is less effective in dissolving flavonoids [10].

Standardization test of jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract

The result of standardization test of jackfruit (Artocarpus heterophyllus Lamk.) leaf extract was described on Table 2.

Table 2. Assay results of Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract.

Parameters	Results	References
Specific parametes		
Color	Dark green	
Aroma	Aromatics	
Taste	-	
Shape	Condensed extract	
Water soluble and ethanol soluble compounds	59.245%	< 100%
Phytochemical screening		
Alkaloids	Negative	
Flavonoids	Positive	
Saponins	Positive	
Tanins	Positive	
Steroids/Trierpenoids	Negative	
Non-specific parameters		
Total ash content	7.85%	<15%
Acid-insoluble ash content	0.23%	<1.5%
Loss on drying	1.43%	<10%

Physical quality test of serum of jackfruit (*Artocarpus heterophyllus* Lamk.)

The result of physical quality test of serum of jackfruit was described on Table 3. The organoleptic of leaf serum is dark green, leaf aroma, and is slightly liquid. The pH test is crucial for assessing the acidity or alkalinity of topical products to ensure they do not disrupt the skin's balance. The alkaline-pH scale, which ranges from 4.5 to 6.5, is sensitive to changes in pH and can help detect dryness. The pH test results indicate that the 4% serum has a pH of 5.48 and the 5% serum has a pH of 5.22, both of which fall within the skin's natural pH range [13].

Table 3. Results of physical quality test of serum of Artocarpus *heterophyllus* Lamk.

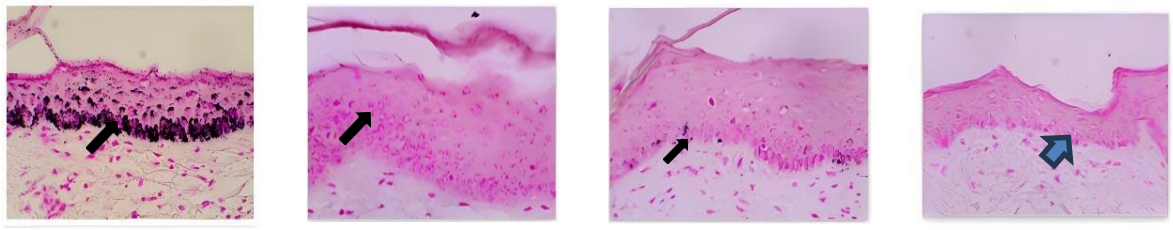
Organoleptic Parameters	**Results**		
Color	Dark green		
Aroma	Typical Jackfruit		
Taste	-		
Shape	a bit liquid		
Parameters	**Results**		
	Serum 4%	Serum 5%	Serum base
pH	5.48	5.22	4.98
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Spreadability	6.2	6.93	6.23
Stickiness	1.58	2.54	1.24
Viscosity	213.3cPs	288 cPs.	

Homogeneity testing ensures the quality of cosmetic preparations by checking for even mixing of active substances and other ingredients. The aim is to ensure that the preparation is free of coarse particles and unevenness. In this study, the serum preparation formulation was homogeneous [22]. The results of spread ablity show that requirements are met with an optimal diameter of 5 to 7 cm, ensuring smooth and even application.[15]. The results of stickiness showed that all formulas met the criteria of adhesion time of more than 1 second, which has an impact on therapeutic effectiveness and drug absorption. The adhesion test measures how long the serum stays on the skin, with higher values indicating a better ability to adhere and absorb [14].

Viscosity tests are important for evaluating cosmetic products as they indicate flow, dispersion and comfort of use The test results revealed that the viscosity of the 4% serum was 213.3 cPs and the 5% serum was 288 cPs, both of which fall within the standard viscosity range for serums, which is between 230 and 1150 cPs.. Lower viscosity improves comfort and ease of use [15].

Antihyperpigmentation effectiveness test results

The anti-hyperpigmentation of effectiveness was shown on figure 1, the histology of melanin. This figure of negative control shown the skin of guinea pigs treated with only the serum base (placebo), without any active ingredient. The dense dark pigmentation seen along the basal layer of the epidermis (highlighted by the black arrow) indicates a high melanin concentration. This result reflects the natural hyperpigmentation induced by repeated UV-A exposure without any therapeutic intervention. The figure of positive control represents the standard treatment group using hydroquinone serum, a commonly used depigmenting agent. The arrow indicates a visibly reduced amount of melanin deposition compared to the negative control, confirming hydroquinone's effectiveness in inhibiting melanogenesis and lightening the skin. In the treatment with 4% *Artocarpus heterophyllus* leaf serum, shown the melanin deposition, indicated by the arrow, appears reduced compared to the negative control but is still slightly more prominent than in the hydroquinone group. This suggests that the 4% serum has moderate anti-hyperpigmentation effect. The treatment with 5% *Artocarpus heterophyllus* leaf serum shown the arrow points to a markedly reduced melanin presence, like or slightly better than the hydroquinone group. This supports the conclusion that the 5% formulation is more effective at reducing melanin synthesis and accumulation, likely due to higher concentrations of flavonoids and antioxidant activity.



(a) (b) (c) (d)

Figure 1. Histology of melanin. (a) Negative Control Group; (b) Control Group (Cos De Baha® Serum, South Korea); (c) Treatment with 4% *Artocarpus heterophyllus* Leaf Serum; (d) Treatment with 5% *Artocarpus heterophyllus* Leaf Serum.

Proposed mechanism of action of Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum

Exposure to UV light can induce pigmentation by several mechanisms, such as increasing the activity of melanogenic enzymes, causing damage to DNA that stimulates melanogenesis, facilitating the transfer of melanosomes to keratinocytes, as well as increasing the activity of melanocyte dendritic cells. UV rays can also trigger the production of ROS which triggers the release of melanocyte stimulating hormone (MSH) and PGE2, which stimulate the process of melanogenesis and melanin production by melanocytes [23].

The anti-hyperpigmentation effect of Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum, as shown in Figures 1a to 1d, is primarily attributed to the presence of flavonoids, polyphenols, and antioxidant compounds in the leaf extract. These bioactive compounds act through several synergistic mechanisms. First, inhibition of melanogenesis via tyrosinase suppression. Flavonoids, which are abundant in jackfruit leaves, serve as potent inhibitors of tyrosinase—an essential enzyme in melanin biosynthesis. By inhibiting tyrosinase and DOPA oxidase activity, these compounds prevent the enzymatic conversion of tyrosine to melanin. This mechanism underlies the reduced melanin accumulation observed in the histological images, particularly in the group treated with 5% serum (Figure 1d), highlighting the serum's dose-dependent depigmenting effect. Second, antioxidant activity to scavenge ROS. Exposure to UV-A radiation generates reactive oxygen species (ROS), which play a significant role in melanogenesis by activating melanocyte-stimulating pathways. The flavonoids and polyphenolic antioxidants present in jackfruit leaf extract neutralize ROS, thereby disrupting the oxidative cascade that stimulates melanin production. In addition to their anti-melanogenic effect, these antioxidants protect epidermal cells from UV-induced damage, supporting a healthier skin barrier and reducing pigmentation. Third, modulation of MITF pathway. Flavonoids may also exert their effect by downregulating the expression of microphthalmia-associated transcription factor (MITF), a critical regulator of melanocyte development and melanin synthesis. Suppression of the MITF pathway results in decreased transcription of melanogenic enzymes, including tyrosinase, further contributing to the inhibition of melanin production.

Calculation of the amount of melanin

Melanin data was measured using the ImageJ application from images of guinea pig skin. It shown on Table 4. By qualitative, visible differences in melanin between negative control, positive control, and two serum treatment groups with extract.

Table 4. Melanin analysis results.

Group	N	Mean ± SD	F	P
Negative Control	3	17.9667±1.67730		
Positive Control	3	1.1667±0.28290		
4% treatment	3	3.5600±2.77422	55,576	0,00
5% treatment	3	2.5633±1.25053		

On average, the percentage of melanin in 4% Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf. serum was 3.5600 ± 2.77422 and 5% *Artocarpus heterophyllus* Lamk. serum was 2.5633 ± 1.25053. A higher concentration of

the extract leads to a lower percentage of melanin, demonstrating its effectiveness in inhibiting melanin production. The melanin results from the three treatments were compared with the negative control. Both Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum concentrations showed a significant decline in the amount of melanin compared to the negative control, showing that Jackfruit *(Artocarpus heterophyllus* Lamk leaf. serum has its own anti-hyperpigmentation effect. In fact, the second serum concentration of Jackfruit *(Artocarpus heterophyllus* Lamk. *leaf* showed the same anti-hyperpigmentation effect with the positive control (2% hydroquinone serum). 5% Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum was proven to have a better anti-hyperpigmentation effect compared to 4% *Artocarpus heterophyllus* Lamk. serum. Jackfruit (*Artocarpus heterophyllus* Lamk serum is known to contain flavonoids as antioxidant compounds which have a mechanism for inhibiting melanogenesis through the microphthalmia-associated transcription factor (MITF) pathway [24]

Epidermal hyperplasia test result

Epidermal hyperplasia test result shown on Figure 2. The image of negative control group shows the skin of the group that received no active treatment—only the serum base. The arrow points to significant thickening of the epidermis (103.24 µm), indicating pronounced epidermal hyperplasia caused by UV-A exposure. The thickened layer is a response to oxidative stress and free radical damage that stimulates abnormal cell proliferation. The figure of positive control group (hydroquinone serum) represents skin treated with hydroquinone serum, the standard depigmenting agent. The epidermis is visibly thinner than in the negative control group, indicating suppressed hyperplasia. Hydroquinone not only reduces melanin but also has antioxidant properties that protect epidermal structure from further damage. The treatment with 4% *Artocarpus heterophyllus* leaf serum shows moderate improvement, with reduced epidermal thickness (average: 72.66 µm) compared to the negative control. The arrow highlights decreased hyperplastic layers. This result suggests that the antioxidants in the 4% serum formulation help mitigate UV-A-induced cellular damage, albeit to a lesser extent than higher concentrations. The treatment with 5% *Artocarpus heterophyllus* leaf serum presents the best therapeutic outcome among the treatment groups. The epidermis is much thinner (average: 44.84 µm) and more comparable to healthy skin. The antioxidants and flavonoids in the 5% jackfruit leaf serum effectively combat oxidative stress, reducing inflammation and cell proliferation. This confirms its strong anti-inflammatory and skin-repairing effects, reversing the hyperplastic response to UV-A exposure.

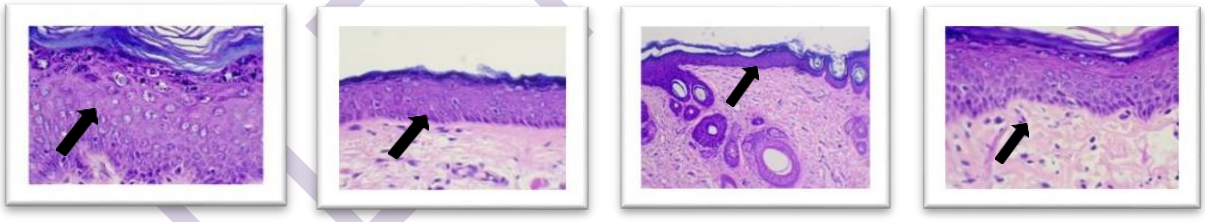
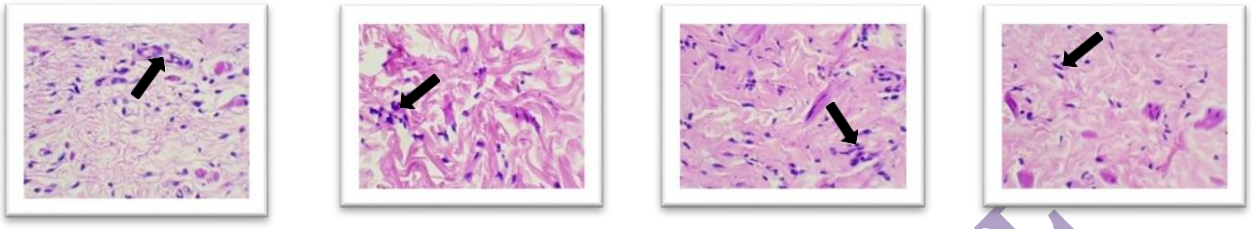


Figure 2. Epidermal hyperplasia features (Hematoxylin–eosin staining). (a) Negative Control Group; (b) Positive Control Group (Cos De Baha® Serum, South Korea); (c) Treatment with 4% serum; (d) Treatment with 5%serum.

Results of fibroblast count test

The results showed in Figure 3. The image of negative control group represents the group treated with only the serum base (placebo), without any active ingredient. The arrow points to a sparse population of fibroblast cells, with a count of 20.33 cells. This low number reflects the skin's poor regenerative response and damage due to oxidative stress caused by UV-A radiation. The lack of active compounds means minimal stimulation of fibroblast activity. The positive control group (hydroquinone serum) shows a moderate increase in fibroblast density, with a count of 28.00 cells.. This suggests that hydroquinone may offer some degree of dermal repair support beyond its depigmenting action, although it's not its primary function. The treatment with 4% *Artocarpus heterophyllus* leaf serum shows improved fibroblast presence (42.67 cells), indicating that the serum at 4% concentration stimulates a regenerative response in the skin. The increase is likely due to the flavonoids and tannins found in jackfruit leaves, which promote fibroblast proliferation and

collagen synthesis by reducing lipid peroxidation and neutralizing free radicals. The treatment with 5% *Artocarpus heterophyllus* leaf serum shown the most significant increase in fibroblast count is seen here, with an average of 54.67 cells. The arrow highlights the abundant fibroblast activity. This result shows the strong regenerative potential of the 5% serum, confirming that a higher concentration of antioxidants and polyphenolic compounds enhances tissue healing, supports capillary formation, and improves structural integrity of the dermis.



(3a) (3b) (3c) (3d)

Figure 3. Overview of fibroblast count (hematoxylin–eosin staining). (a) Negative Control Group; (b) Positive Control Group (Cos De Baha® Serum, South Korea); (c) Treatment with 4% serum; (d) Treatment with 5%serum.

Total flavonoid assay

Flavonoid analysis was performed by UV-Vis spectrophotometry. Determination of flavonoid content of Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract using $AlCl_3$ colorimetric complex method. From measuring the absorbance of quercetin standard solutions (20-100 ppm), a linear regression equation y = 0.0069x + 0.0425 was obtained, with a correlation coefficient r^2 of 0.9949. The results showed on Table 5 that serum flavonoid concentrations in 4% and 5% samples were 1.902 g/100g and 10.5942 g/100g, respectively. Total flavonoids analysis, uses the quercetin standard (g/100g) as a reference in analyzing the total levels of flavonoids contained in the serum Artocarpus heterophyllus Lamk. 4% and 5%.

Table 5. Total flavonoid assay results.

Sample	Results
Serum of *Artocarpus heterophyllus* Lamk. 4%	1.902 g/100g
Serum of *Artocarpus heterophyllus* Lamk. 5%	10.5496 g/100g

Antioxidant activity assessment using the 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) method

Antioxidant activity was shown on Table 6. From the test result indicates that the antioxidant activity of Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves are lower than vitamin C, possibly due to the duration of maceration which affects the IC_{50} value. Flavonoids exhibit antioxidant mechanisms by directly capturing ROS, preventing their regeneration, and indirectly enhancing the activity of cellular antioxidant enzymes. Flavonoids are antioxidants that function to protect lipophilic antioxidants, so they can strengthen cellular antioxidants. Antioxidant activity of Jackfruit (*Artocarpus heterophyllus* Lamk.) was performed using a UV-Vis spectrophotometer at wavelengths of 400-800 nm. This test uses DPPH, which is a warning signal from purple to pale yellow color when in contact with antioxidants. Vitamin C was used as a comparator. As a result, Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract showed Inhibition concentration 50 of 132.934 ppm (4%) and 40.930 ppm (5%), while vitamin C produced IC50 of 3.023 ppm. The classification of antioxidant potential is as follows: Very low (IC50 <50 ppm), medium (IC50 between 51 and 100 ppm), high (IC50 between 101 and 150 ppm), low (IC50>151 ppm), and non-active (IC50>500 ppm) [25].

Table 6. Dpph test results.

Sample	Results of IC_{50}
Serum of *Artocarpus heterophyllus* Lamk. 4%	132.934 ppm
Serum of *Artocarpus heterophyllus* Lamk. 5%	40.930 ppm
Vitamin C	3.023 ppm

▪ CONCLUSION

Specifically, this study showed that Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum at 4% and 5% concentration was effective in reducing the amount of melanin in a guinea pig model of melasma caused by UVA light exposure. The serum showed comparable results to hydroquinone serum, which is the gold standard in melasma therapy, but with minimal side effects. In general, this study proved that Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf extract has great potential as a natural anti-hyperpigmentation agent. Jackfruit (*Artocarpus heterophyllus* Lamk.) leaf serum not only meets good physical quality standards but also provides a safer and more effective solution to treat melasma compared to the use of synthetic chemicals. These results support the use of natural ingredients, especially Jackfruit (*Artocarpus heterophyllus* Lamk.) leaves, as a potential alternative in cosmetics and dermatology for the treatment of skin hyperpigmentation due to UVA light exposure.

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