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Research Article
Effectivity of Gel Derived from Degumming Silkworm Cocoon Waste for Skin Pigmentation

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Abstract

Background and Objective: The trash produced by boiling silkworm cocoons during the degumming process still retains useful amino acids, such as sericin and fibroin, that can repair skin damage caused by exposure to ultraviolet light. This study seeks to examine the impact of utilising degumming silkworm cocoons (DSC) gel, derived from boiling waste, on the healing process of rat skin that has been subjected to Ultraviolet (UV) light, as well as the determination of the Sun Protection Factor (SPF) value. **Materials and Methods:** The research used a Completely Randomized Design (CRD) consisting of 5 treatment groups, namely the positive control, avobenzone, the group without avobenzone and DSC gel and the administration of DSC gel at 20, 40 and 60% for 14 days. The research used 25 male rats (*Rattus norvegicus*). Skin tissue was prepared for microscopical examination using the paraffin method combined with Hematoxylin and Eosin (H&E) staining. The data were analyzed statistically with the one-way ANOVA test and continued with the *post hoc* Duncan's test. Non-parametric data were carried out by the Kruskal Wallis test and continued with the Mann Whitney test. **Results:** The degumming silkworm cocoons (DSC) gel had a fairly high SPF value with an extra protection category of 7.10 at a concentration of 60%. The DSC gel had the potential to accelerate the recovery of skin as observed by the test subjects showing no wrinkles and redness. An increase in epithelial thickness and a decrease in melanocyte cells were also obtained from the treatment. Increasing the concentration of DSC gel also improved the skin recovery exposed to ultraviolet light. **Conclusion:** The effective and efficient concentration of cocoon degumming waste gel is 40-60%. The ability of silkworm cocoon degumming waste to rejuvenate UV-exposed skin suggests its future application as a topical preparation for promoting skin health.

Key words: Degumming waste gel, melanocytes, silkworm cocoon, ultraviolet light, sun protection factor, *Bombyx mori*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The silkworm cocoons (*Bombyx mori*), in addition to producing silk thread, also contain specific proteins such as fibroin and sericin[1,2]. Research on the protein indicates that it has application potential in the food, cosmetics, biomaterials and polymer sectors. Silkworm cocoons contain natural compounds or flavonoids because silkworms consume mulberry plants (*Morus* sp.) which also contain similar compounds[3].

The degumming procedure is used to produce cocoons from which silk thread can be made. Boiling silkworm cocoons creates waste in the form of boiling water, which is then used in the degumming process to produce boiling water which also includes sericin protein[4]. Sericin is useful as an antioxidant, anti-inflammatory, antiapoptotic, moisturizer, wound healing, antibacterial, antitumor and protection against Ultraviolet (UV) radiation[5]. Sericin as textile waste, can be used for antioxidant and skin whitening purposes. With its high iron-reducibility, phenolic acid content, flavonoids and tyrosinase inhibitory action, vitamin C has the highest antioxidant activity. It also can skin whitening properties due to its high tyrosinase activity[6]. Ultraviolet (UV) light is one of the rays emitted by the sun that can harm the skin, including damage to skin cells, the occurrence of wrinkles, uneven skin color and texture, redness or erythema and pigmentation[7]. The lack of melanocyte cell counts from normal limits is the cause of abnormal skin color.

The time of exposure, intensity and wavelength of sunlight that is exposed to the skin all affect how much damage it sustains. Skin is naturally resistant. When exposed to UV radiation, the skin will thicken the stratum corneum and increase the size of the melanin filter in the epidermis. The protective system cannot survive solar radiation if the skin is exposed to too much radiation. Sunscreen is therefore, a necessary extra kind of protection[8]. There are several different dose forms for sunscreen, including lotion, cream, spray, sticks and gel. The best preparation to use is a gel since it can be absorbed by the skin more readily[9]. In this study, a preparation in the form of a gel made from degumming waste (boiling) of silkworm cocoons was used.

There has been a lot of research on the use of silkworm cocoon boiling waste as a cosmetic ingredient, but it has only focused on making preparations without *in vivo* application. As a result, more study is needed to determine whether the gel made from degumming waste of silkworm cocoons can improve skin pigmentation as a sunblock.

MATERIALS AND METHODS

Study area: The research project was conducted from March, 2021 to August, 2021. The research was carried out in the Animal House and Microbiology Lab, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Medan, Indonesia.

Animal handling: A total of 30 Wistar rats (*Rattus norvegicus*) were obtained from the Animal Physiology Laboratory, Biology Department of the University of North Sumatra for the experiment. The animals were cohabitated in groups of four within a single enclosure and kept under controlled conditions of temperature and humidity. They were subjected to a chronobiological regimen consisting of alternating 12 hrs periods of light and darkness. All animals were supplied with rat food and water as required. The research was conducted following the acquisition of an ethical permit for conducting experimental animal research (Number: 0231/KEPH-FMIPA/2021).

Research design

Experimental design: This research was conducted using a Completely Randomized Design (CRD) method, with 5 treatment groups exposed to ultraviolet light with a wavelength range of 290-320 nm, because it was equivalent to the wavelength of UV-B light[10] and 5 replications. The test animals used were 25 male white rats (*Rattus norvegicus*). The rats were 6-8 weeks old and had a body mass of ±150 g. This study used 5 treatments, namely: K+: UV exposure and administration of commercial sunscreen (avobenzone), K-: Without sunscreen application, P1: Administration of 20% silkworm cocoon degumming waste gel, P2:40 and P3:60%.

Degumming process of silkworm cocoons: The cocoons were cleaned from floss and pupae remnant, then the cocoons were washed with clean water and dried. Boiling was carried out using an autoclave at a temperature of 121°C, 2 atm, for 60 min, with a ratio of 30:1, which was 300 mL of distilled water and 10 g of cocoons[11].

Preparation of degumming silkworm cocoons gel from boiling waste: The degumming silkworm cocoons (DSC) gel was prepared from the boiling waste of silkworm cocoons mixed with the gel base[12]. Methylparaben was dissolved in distilled water, homogenized, then mixed with triethanolamine. Carbopol® 940 polymer was dissolved in

warm water, then crushed to form a gel texture using a mortar and pestle and left for 1 hr. Glycerin was added to carbopol 940 which had been slowly developed and stirred until homogeneous. The cocoon-degumming waste solution was added and then homogenized again.

Treatment stage: Rats were placed in cages based on their groups, acclimatized for 7 days and fed *ad libitum*. After completion of acclimatization, each rat was given a treatment. The hair on the dorsal part was shaved with a sterile razor. The duration of UV exposure was 30 min with the distance between the lamp and the rats as far as 15 cm. The wavelength used is equivalent to the wavelength emitted by UV-B light, which is 290-320 nm[12]. On K+, the commercial sunscreen gel or avobenzone was applied and exposed to UV; on K-, the rats were exposed to UV light without any gel administration; on P1, the rats were applied with 20% DSC gel and exposed to UV light; on P2, the rats were applied with 40% DSC gel and exposed to UV light and on P3, the rats were applied with 60% DSC gel and exposed to UV light. The silkworm cocoon degumming waste gel was given 2 times a day, i.e., 20 min before exposure to UV light and 4 hrs after exposure to UV light. The UV exposure was carried out 3 times a week for 2 weeks. The gel was still given on days without irradiation. Rats were killed via cervical dislocation and after the skin organs were cut into 2×2 cm^2, preparations were finished using the paraffin technique.

Determination of Sun Protection Factor (SPF): The SPF value can be determined by using UV-Vis spectrophotometry. The cocoon degumming waste gel was diluted to 4000 ppm and then the absorbance was calculated at 290-320 nm at 5 nm intervals, with distilled water as the blank as a solvent. The SPF value was calculated as:

$$SPF = CF\times\sum_{290}^{320} EE\ (\lambda)\times I\ (\lambda)\times Abs\ (\lambda)$$

where, CF is correction factor (10), EE (λ) is erythmogenic effect of radiation with wavelength λ, Abs (λ) is spectrophotometric absorbance values at wavelength λ. The values of EE×I (λ).

Morphological observation: Morphological observations were carried out each day for 14 consecutive days, by observing the skin visual appearance in each treated area. The wounds were visually observed by indicating irritation (dryness, peeling skin), erythema or redness and wrinkles. Observations will be scored: (0) Complete recovery (moist skin and new hair growth), (1) Dry skin and or redness, (2) Skin has wrinkles, (3) Skin has wrinkles and or redness and (4) Dry and flaky skin, redness and wrinkles.

Histological observation: Histological observations were carried out by observing the number of melanocytes and the thickness of epithelial tissue on the preparations using a light microscope (Olympus CX 23) with a magnification of 400 times and photographed with an AmScope MU130 camera connected to a computer monitor. The calculation is carried out in as many as 5 fields of view. The thickness of the epithelial tissue can be determined by measuring its thickness, then adding up the thickest layer with the thinnest layer and averaging it. The number of melanocytes was counted manually with the help of Image J software.

Statistical analysis: Data were analyzed for statistical difference using SPSS version 22.0. Morphological data were analyzed using the Kruskal-Wallis test at $p\leq0.05$, followed by a *post hoc* analysis, the Mann-Whitney test. Histological data were analyzed using the ANOVA test at $p\leq0.05$, followed by a *post hoc* analysis, Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The results of the SPF value obtained from silkworm cocoon degumming waste gel with three levels of concentration, namely at a concentration of 20% has an SPF value of 3.45 with a minimum of 40% has an SPF value of 5.51 with moderate and 60% had an SPF value of 7.10 with extra protection category (Table 1).

Morphological observation on UV-exposed skin: The condition of rat skin that has been exposed to ultraviolet light causes various reactions, including the presence of wrinkles, redness or erythema and dry and peeling skin surface conditions. On the initial day, after being exposed to ultraviolet light it causes a reaction in the form of redness or erythema, there are wrinkles and the condition of the skin surface is dry. On the 7th day after being exposed to ultraviolet light, the skin condition of the mice was still in the recovery stage. The skin reaction is still dry and there is redness, dry but not peeling. In the P3 group, a small area of the skin has started to grow new hair. The skin condition of the mice on the 14th day had returned to normal with the criteria of moist skin, no wrinkles and redness in the P2 and P3 treatment groups, but in the K- group, the skin conditions were red and dry. In the K+ and P1 groups, redness was still visible which indicated that the skin was still recovering. The condition of the skin that has been exposed to ultraviolet light and given a silkworm cocoon degumming waste gel can be seen in Fig. 1 and 2.

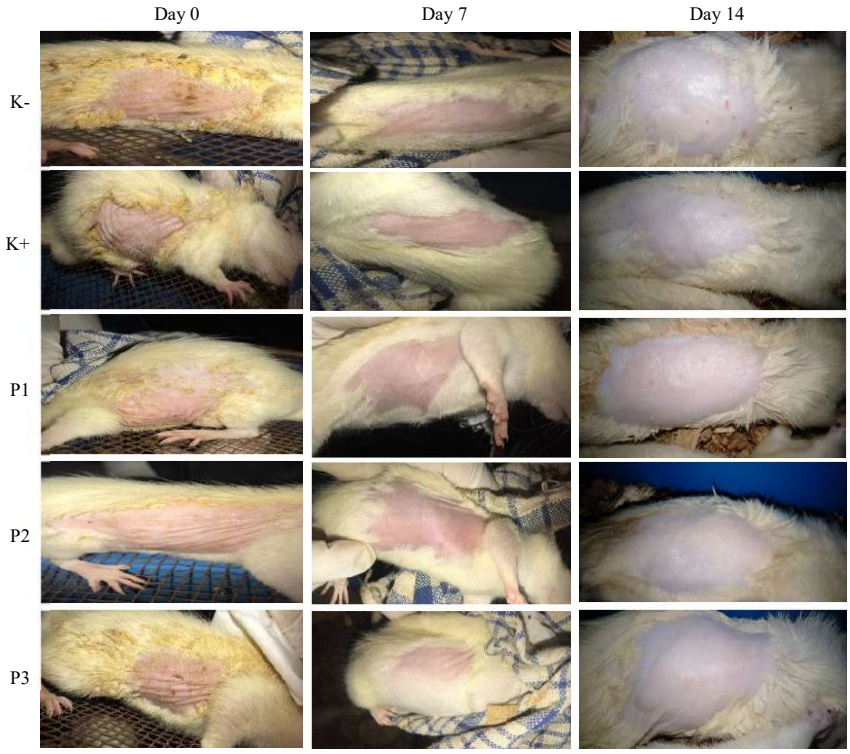

Fig. 1: Visual appearance of untreated group (K-), avobenzone-treated group (K+) and DSC gel- treated group (P1, P2 and P3) after UV exposure

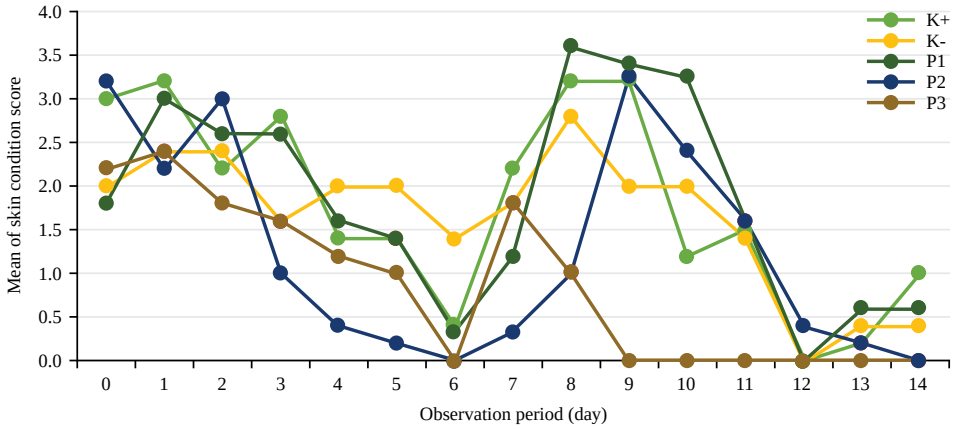

Fig. 2: Skin regeneration performance of control and treated group after UV exposure

Table 1: Sun Protection Factor (SPF) value of silkworm cocoon degumming waste gel concentrations of 20, 40 and 60%

Cocoon degumming waste gel concentration (%)	SPF value	Category
20	3.45	Minimum
40	5.51	Currently
60	7.10	Extra

The average scores of skin conditions from the control and treated groups were presented in Table 2 and were tested statistically. The results showed that the avobenzone-treated group was not significantly different with K-, P1 and P2 which indicated a longer period for skin to rejuvenate. The P3 group was categorized as the best treatment based on its skin condition score among the groups.

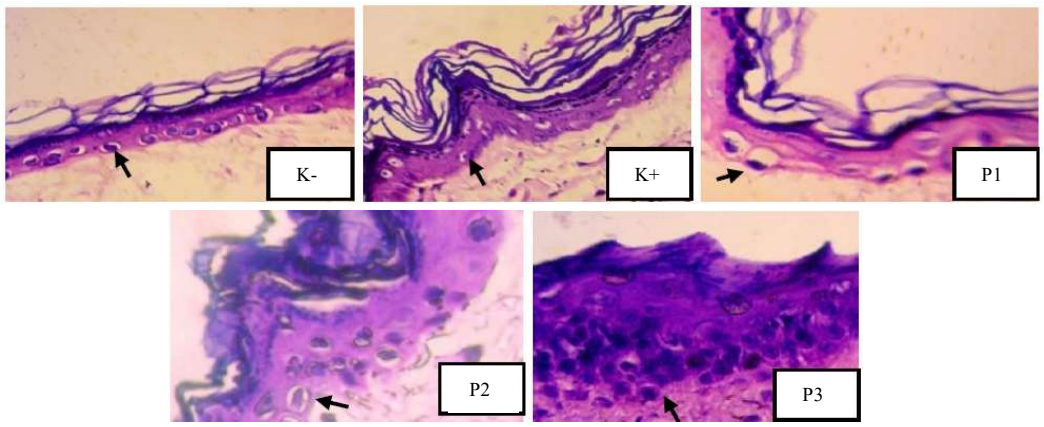

Fig. 3: Histological image of H&E stained-skin tissues showing several melanocytes in control and treated groups
Magnification at 20×

Table 2: Skin condition score of control and treated group using DSC gels

Treatment group	Average of skin condition score
K-	24.6±11.89^{bc}
K+	26.6±11.97^{bc}
P1	26.8±9.26^{c}
P2	17.8±2.77^{b}
P3	13.0±2.35^{a}

Value with similar letters in the same column indicate no statistical difference at $p \leq 0.05$

Table 3: Thickness of epithelial tissue and melanocyte count on control and treated group

Treatment group	Thickness of epithelial tissue (μm)	Number of melanocytes
K-	57.424±1.58^{a}	22.6±13.35^{b}
K+	67.420±9.43^{a}	11.6±2.23^{a}
P1	147.620±4.64^{b}	11.0±4.65^{a}
P2	176.920±6.78^{c}	7.8±4.71^{a}
P3	285.198±24.81^{d}	4.0±2.34^{a}

Value with similar letters in the same column indicate no statistical difference at $p \leq 0.05$

Thickness of epithelial tissue and number of melanocytes on UV-exposed skin: The skin conditions as indicated from the thickness of epithelial tissue and the number of melanocytes were presented in Table 3. In general, the application of DSC gels gave an increase in the thickness of the UV-exposed skins ($p \leq 0.05$). The histological appearance that showed the number of melanocytes was presented in Fig. 3. The highest number of melanocytes was observed in the K- group while the lowest was in P3 (60% of DSC gel).

Sun Protection Factor (SPF) properties of DSC gel: The SPF value of DSC gel using three different concentrations (20, 40, 60%) resulted in different effectivity. The highest SPF value was obtained at 7.10 or categorized as extra protection from the highest DSC gel concentration (60%), followed by 40% with an SPF value of 5.51 or moderate protection and 20% with 3.45 or minimal protection. The higher the concentration DSC gel gave the higher the SPF value. However, the result was still inferior compared to avobenzone with an SPF value of 30 or ultra protection. Previous studies have reported the SPF value of other natural compounds such as floral and leaf extract of marigolds at 10 and 15, respectively[13], coconut water at 7.38 and watermelon extract at 0.97[14].

Dutra *et al.*[15] stated that the SPF value is being used as a universal indicator to assess the effectivity of a product or compound(s) that may act as a UV-protection agent. Based on the suggestion by the Food and Drug Administration, a recommended UV-protection agent should have an SPF value from 15 to 50. The level of SPF in sunscreen was determined by the time it takes for sunburn to appear on the skin. The SPF value indicates how long the skin remains protected from sun exposure.

Morphological observation on UV-exposed skin: Based on Fig. 1, the administration of 60% DSC gel gave the best result in preserving the skin condition of treated rats (P3). The skin part of the P3 group was observed to regenerate faster in each day of observation. However, the regeneration ability of applied gels was still fluctuating due to the presence of a score of 3 with the criteria of skin condition having wrinkles and or redness and a score of 4 with the criteria of skin condition having wrinkles, redness or erythema, as well as dry and flaky skin surface conditions. This is caused by repeated exposure to UV rays, so some treatment groups whose skin conditions are not fully in normal condition, respond again after irradiation. The P3 treatment group had recovered since the ninth day, despite repeated irradiation.

Figure 2 shows that on the first day after being exposed to ultraviolet light, all treatment groups had an average value that tended to be high. On the next day, the P2 group experienced a decrease, while the other group experienced an increase compared to the skin conditions on the first day. On day 9, the K+ group increased again, but the P1 and P2 groups were stable. This indicates that the condition of the skin with K+ treatment was not better than the previous day, while the P1 and P2 groups showed that the skin condition was not getting worse and gradually recovered, although on 3 days before the end of the observation there was an increase again. The P3 treatment group showed that the condition of the skin exposed to ultraviolet light had returned to normal and had not been damaged again until the last day of observation.

Following UV exposure, the skin's healing process is influenced by several variables, such as the animal's health (i.e., whether or not it is stressed out during treatment), the environment, repeated exposure and the efficacy of a product containing specific active ingredients to help accelerate healing. Data indicates that the administration of DSC gel at 60% recovered faster because of the high levels of functional protein contained in the gel, thus the sericin content contained also more. This suggests that the active ingredients present in a particular substance also affect the rate of recovery of the skin condition.

The protein in silkworm cocoons and its remnant during the process of degumming is suitable to be applied in pharmaceutical or cosmetic products, particularly as sunscreening agents[6]. The sericin concentration in silkworm cocoons was up to 30%, yet the incorporation of silkworm material into cosmetic products in higher concentrations may improve its bioactivity[5].

The skin will become red a few hours after exposure to UV rays and reach a peak 8-12 hrs later, then slowly return to normal[16]. Erythema is the term for this redness' outward manifestation. Erythema, sometimes referred to as tanning, is a disorder where the skin goes from red to dark. After radiation exposure, this is the primary skin reaction that manifests. The degree of the erythema that develops serves as a reference signal for the degree of skin epidermal damage. The appearance of a reddish tint on the skin's surface, the development of wrinkles and finally dry, peeling skin as a symptom of the exfoliation of dead skin cells, are the three phases of erythema or redness on the skin.

Thickness of epithelial tissue and number of melanocytes on UV-exposed skin: The thickening of epithelial tissue is a natural protection effort by the skin and assistance from the provision of substances containing active ingredients that have the potential to protect the skin from UV rays. The increase in the thickness of the epithelial tissue on the 14th day of observation, even though it was heading towards a normal skin condition, was histologically still in the recovery stage. The thickness of the epithelial tissue will continue to increase to the normal limits of skin elasticity, but in some cases some exceed the normal limits, resulting in the formation of scar tissue.

The stratum corneum thickening process and the production of melanin pigment from melanocyte cells are the two natural defense systems of the skin[17,18]. The UV rays trigger the thickening of the stratum corneum up to three times. The thickened stratum corneum will absorb, scatter and reflect the ultraviolet light. The epidermis layer of thick skin will provide maximum protection and has a higher sensitivity and tolerance to ultraviolet light exposure than parts of the skin that have a thin epidermal layer. When the skin is exposed to UV rays, there will be damage to the stratum corneum layer. To restore the skin, cells in the outermost layer of the skin will respond to the damage by increasing their mitotic activity, proliferation and maturation of several basal layer cells. This will speed up the keratinization process that is brought on by UV exposure.

Measurement of epithelial tissue thickness was carried out with the upper limit in the form of keratin fibers to the most basic layer of the epidermis, namely the stratum germinativum. The cells that make up the stratum corneum are cells whose core organelles are missing and the cytoplasm is filled with scleroprotein filaments, called keratin. The surface area depends on age, anatomic location and conditions affecting epidermal proliferation such as UV irradiation. The main function of the epidermis is the production of the stratum corneum (SC) which protects our body from drying out as well as from invading pathogens[19]. There are two compartments of the corneocyte system, the flattened dead cell body of the keratinocyte covered by a strong and highly crosslinked protein coat and the extracellular lipid lamellae[20].

Based on the results, the application of DSC gels may indicate its whitening properties to the skin. The low number of melanocytes indicated the low incidence of skin pigmentation which shows its effect on protecting the skin environment. The lowest layer of the epidermis is the basal layer or stratum germinativum which consists of melanocyte cells that function as pigment formation. The presence of melanocyte cells is one of the skin's natural protection efforts from ultraviolet radiation[16,18,21]. Keratin and proteins in the stratum corneum diffuse and absorb UV rays. Keratinocytes respond to UV light by increasing the production of ET-1 and POMC, the increased production of ET-1 and POMC causes a new demand for melanosomes. Melanosomes produce and release melanin from melanocytes to keratinocytes. Melanocytes are located in the epidermis, part of the stratum germinativum. The skin sensitivity is determined by the number of melanocytes. Melanin units of the epidermis are formed when melanocytes interact with 30-40 keratinocytes and Langerhans cells[22]. Melanocyte dendrites can migrate and have a variable size depending on the demands of a certain place. They are extended between the surrounding keratinocytes. Exposure to sunlight is one of the elements that influence the formation of melanosomes[23].

CONCLUSION

The effectivity of DSC gel to preserve the skin condition or skin pigmentation in rats was achieved from the application of 60% concentration based on its high SPF value, the thickness of epithelial cells and low number of melanocytes. The DSC gel may be further investigated for its safety and compatibility with human skin.

SIGNIFICANCE STATEMENT

This study discovers that silkworm cocoon degumming waste has the potential to restore skin exposed to ultraviolet radiation and can be applied as a topical preparation. It can as herbal for skin problems in histology. This study will help the researcher uncover the role of silkworm cocoon degumming waste for drug development in healthy skin. Thus, a new theory on the role of the degumming waste in skin therapy in humans may be arrived at.

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