



## Protective Potential of *Garcinia Cowa* Roxb Fruit Peel Extract Against UVB-Induced Skin Damage in *Rattus norvegicus* Rats

Prima Minerva<sup>1\*</sup>, Riesye Arisanty<sup>2</sup>, Rinita Amelia<sup>3</sup>, Shinta Ayu Intan<sup>4</sup>, Maida Wilis<sup>5</sup><sup>1</sup>Department of Cosmetology and Beauty, Tourism and Hospitality Faculty, Universitas Negeri Padang, Padang, Indonesia.<sup>2</sup>Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia<sup>3</sup>Department of Histology, Faculty of Medicine, Universitas Baiturrahmah, Padang, Indonesia<sup>4</sup>Department of Anatomical Pathology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia<sup>5</sup>Department of D3 Nursing, Faculty of Health and Psychology, Universitas Negeri Padang, Padang, Indonesia

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### ABSTRACT

*Garcinia cowa* (*G. cowa*) Roxb., a medicinal plant used in traditional medicine, possesses antioxidant properties and contains bioactive compounds like flavonoids and vitamin C. This study examined the impact of *G. cowa* extract from the fruit rind on skin thickness, the number of fibroblast cells, and collagen density in *Rattus norvegicus* rats exposed to UVB light. Thirty rats were divided into five groups (six each) in a post-test randomized control design. The negative control group (C-) was not exposed to UVB; the positive control group (C+) was exposed to UVB. Three treatment groups (T1, T2, and T3) were exposed to UVB and treated with 3%, 5%, and 8% extracts, respectively, on the back skin. The extract was prepared by macerating 1015 g of the fruit rind with 96% ethanol at a 1:5 ratio for 2 × 24 hours. The results of histopathological analysis revealed several significant changes in each group compared to the control group (C+). Epidermal thickness decreased in all treatment groups: T1 decreased by 37.58%, T2 decreased by 46.22%, and T3 showed the most significant decrease of 58.73%. In contrast, the number of fibroblasts increased in each group: T1 increased by 44.44%, T2 increased by 55.56%, and T3 increased by 102.22%. Similarly, collagen density increased in all treatment groups: T1 by 18.79%, T2 by 9.11%, and T3 by 10.83%. *Garcinia cowa* Roxb fruit peel extract effectively prevents epidermal thickening, protects fibroblast cells from damage, and slows down the loss of collagen density.

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**Keywords:** *Garcinia cowa*, Epidermis, Collagen, Fibroblast, *Rattus norvegicus*, *in vivo*

### Introduction

Skin aging is an inevitable biological process that begins at the onset of an individual's life. This process is not merely a physical alteration associated with aging but rather the outcome of intricate interactions between internal and external elements. Internal variables encompass the body's aging process and hormonal variations that transpire over a lifetime. Simultaneously, extrinsic factors significantly influence outcomes, including exposure to environmental contamination, elevated stress levels, and, predominantly, solar radiation.<sup>1</sup> Long-term exposure to ultraviolet (UV) rays is the primary external factor contributing to premature skin aging, referred to as photoaging.<sup>2</sup> Ultraviolet radiation, particularly UVB rays, can enter the skin and interact with cellular chromophores. This contact releases reactive oxygen species (ROS), highly reactive and unstable chemicals. These ROS subsequently impact the organization and composition of skin cells, leading to structural and functional damage.<sup>3</sup>

\*Corresponding author. Email: [prima.minerva@fpp.unp.ac.id](mailto:prima.minerva@fpp.unp.ac.id)  
Tel: +62-812-7522-5994

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UV rays can cause dual harm to the skin. Initially, it can directly damage the epidermis and dermis, resulting in cellular "burning" and the degradation of essential proteins such as collagen and elastin, which are crucial for skin firmness and elasticity. Second, ultraviolet rays might indirectly impede collagen formation in the skin, thereby exacerbating the photoaging process.<sup>4-5</sup> As a result, photoaging exhibits numerous visible signs, including dry skin, a rough texture, thicker skin, reduced elasticity, dark spots, and wrinkles.

Recently, there has been a significant surge in interest among researchers and the global beauty business in natural solutions to mitigate photoaging. This phenomenon is driven by the rapid growth of the natural skincare cosmetics sector, which is in high demand among consumers. Consistent with this trend, numerous dermatological treatments and topical cosmetic formulations have progressively incorporated natural substances into their products. The conventional use of plants in medicinal and cosmetic applications has established a significant foundation for contemporary research aimed at developing novel natural active ingredients in cosmetics. To meet the growing market demand, it is crucial to explore the potential applications of plant extracts in medicine and skincare products further.<sup>6</sup>

*G. cowa* Roxb. fruit has pharmacological effects as an antioxidant and anti-inflammatory, which is very suitable as a source of natural bioactive substances used in medicine and pharmacy.<sup>7</sup> *G. cowa* Roxb. contains bioactive compounds such as flavonoids, HCA, vitamin C, and vitamin A.<sup>8-9</sup> Vitamin C helps reduce the symptoms of photoaging on the skin and is often used as an active ingredient in anti-aging cosmetics. The antioxidant activity of vitamin C can help minimize the effects of sun (UV) exposure by reducing symptoms of erythema and DNA damage, and preventing premature skin aging.<sup>10-11</sup> Flavonoids can also protect against symptoms of photoaging by providing photoprotection for skin cells against UV radiation, offering antioxidant effects, and

acting as tyrosinase inhibitors that reduce melanin synthesis.<sup>12-14</sup> This occurs as cell aging progresses.<sup>15</sup> *G. cowa* Roxb. shows strong antioxidant properties, which help eliminate free radicals and are crucial for preventing health issues associated with oxidative stress.<sup>16</sup> The plant extracts can help reduce inflammation by lowering the production of pro-inflammatory cytokines like IL-6 and TNF- $\alpha$ , mainly through the NF- $\kappa$ B signaling pathway.<sup>17-18</sup> Antioxidants such as vitamin C, flavonoids, and phenolic acids play an essential role in fighting free radical species, which are the leading cause of various negative changes in the skin.<sup>9</sup> Some of these compounds are found in *G. cowa* Roxb. If proven effective, the plant extracts could be used as a natural skin toner for removing dark spots from the skin and as a natural solution for hyperpigmentation.<sup>19</sup> Although *G. cowa* Roxb has been extensively studied and exhibits a variety of pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, anticancer, and antibacterial properties, based on its rich phytochemical content, no specific studies have examined the potential of its fruit rind extract in addressing UVB-induced skin aging (photoaging). Previous research has highlighted the various health benefits of other parts of *G. cowa* Roxb and their compounds. However, this study focuses on the effects of its fruit rind on key indicators of UVB-induced skin damage. Specifically, the novelty of this study lies in the detailed investigation of the extract's effects on epidermal thickness, fibroblast cell count, and collagen density in a UVB-exposed *Rattus norvegicus* rat model, filling a gap in knowledge regarding the application of *G. cowa* Roxb as a natural anti-photoaging agent. This study examined the impact of *G. cowa* extract from the fruit rind on skin thickness, the number of fibroblast cells, and collagen density in *Rattus norvegicus* rats exposed to UVB light.

## Materials and Methods

### Materials

The materials used were 96% ethanol (Merck), Wistar rats, standard animal feed and water, Hematoxylin (Epredia), and Masson's Trichrome (Scytex). This study used a rotary evaporator (Buchi), a UV B PL S9W/01/2P treatment cage, and scales (Ohaus scales). The *G. cowa* fruits used as samples in this study were derived from Tampunik

Kambang, Pesisir Selatan Regency, West Sumatra, Indonesia (FR5M+FG9), Latitude: -1.8285° and Longitude: 100.9912°. The identification of the samples, done at the Andalas Herbarium at Andalas University, shows that they are *G. cowa* Roxb. ex Chiosy from the Clusiaceae family, and the plant voucher number is 236/K-ID/ANDA/V/2021. The extraction process involves separating the fruit flesh from the peeled skin of *G. cowa* Roxb. and accurately weighing the skin. The ethanol extract of *G. cowa* Roxb. fruit skin was prepared as follows: Fresh tamarind fruit was separated from the skin and flesh, then weighed to yield 1015 g of fresh fruit skin. The purified sample was then extracted by maceration with 96% ethanol (1:5), 2 x 24 hours. The collected macerate was then evaporated using a rotary evaporator until a thick extract was obtained and weighed. The extract was stored at a temperature of 4 °C to 8 °C for further testing.<sup>9-20</sup>

### In vivo study on *Rattus norvegicus* rats

Thirty *Rattus norvegicus* rats weighing approximately 150–200 g under excellent health status were used for this study. The rats were housed in a strictly controlled laboratory environment with a stable temperature of  $23 \pm 1^\circ\text{C}$  and humidity ranging from 50% to 60%. They had unlimited access to drinking water and food in the form of pellets throughout the study period.<sup>16-17</sup> Photoaging was induced by shaving the backs of the rats and irradiating them using a UVB lamp PL S9W/01/2P three times a week for six weeks. Before UVB exposure and the start of treatment, the rats were randomly divided into five groups, each consisting of six rats (Table 1). They underwent acclimatization for one week to adapt to the new environment. The *G. cowa* Roxb extract was administered by applying 0.1 mL/cm<sup>2</sup> to the back skin of the rats twice daily for six weeks. On days of UVB light exposure, the extract was applied for 20 minutes. The extract was applied twice daily on days when they were not exposed to UVB light.

### Post-treatment observations and research ethics

The rats were left for 24 hours after the last UVB to allow the acute effects of irradiation to subside. All the *Rattus norvegicus* rats were humanely sacrificed after the study period for further histopathological analysis. *Ethical approval, number 083/Etik-FK UNBRAH/03/07/2022, was obtained for the study following the seven WHO 2011 standards.*

**Table 1:** Division of model rat groups

Control group		Treatment		
Negative control (C-)	Positive control (C+)	Treatment group 1 (T1)	Treatment group 2 (T2)	Treatment group 3 (T3)
Without exposure to UVB or extracts	Only exposed to UVB light	Exposed to UVB light and given 3% <i>G. cowa</i> Roxb extract	Exposed to UVB light and given 5% <i>G. cowa</i> Roxb extract	Exposed to UVB light and given 8% <i>G. cowa</i> Roxb extract.

### Histopathology of the skin

A series of careful steps in histopathological examination was conducted to analyze the impact of the treatment on the skin of *Rattus norvegicus* rats. Firstly, the biopsied back skin tissue was immediately fixed by immersing it in 10% neutral buffered formalin. This fixation process aims to preserve cellular and tissue structures, thereby preventing cellular and tissue degradation. After fixation, tissue slices were prepared and stained using the hematoxylin and eosin (H&E) staining method. HE staining is crucial for visualizing and measuring the thickness of the epidermis, as well as counting the number of fibroblast cells, which are the primary cells responsible for collagen synthesis in the dermis. Additionally, Masson's trichrome staining was employed to specifically identify and assess the density of collagen fibers, which are important indicators of skin health. The thickness of the epidermis was measured on HE-stained samples using an Olympus BX51 light microscope connected to a PC monitor. The thickness at a magnification of approximately 100x was measured from five different locations (representing 100  $\mu\text{m}$ ) on each slice of the rat skin, determining the vertical distance between the basal epidermal layer and the base of the stratum corneum. The measurement results were presented as the mean and standard deviation of the skin tissue slices

from six *Rattus norvegicus* rats in each group. This analysis process was carried out in a double-anonymized manner, meaning that neither the observer nor the researcher knew the treatment group of the sample to minimize bias.

For the calculation of the number of fibroblast cells, an Olympus BX51 light microscope with a magnification of 400x per field of view was used. The number of fibroblast cells in the skin's dermis was quantitatively calculated. The fibroblasts were randomly counted in each preparation in five fields of view. To simplify and standardize the calculations, the ImageJ software was utilized, arranged in a 255-pixel grid, representing a tissue area of 50  $\mu\text{m} \times 50 \mu\text{m}$ . This method enables the accurate counting of fibroblast cells in HE-stained samples. UVB exposure is known to damage skin collagen, causing a decrease in collagen density. Therefore, Masson's trichrome (MT) staining was used in this study to visualize and assess the effect of *G. cowa* Roxb on collagen integrity. MT staining imparts a blue hue to collagen fibers, allowing clear visualization under the microscope. Collagen density images were obtained using a light microscope equipped with Optilab, producing 400x magnification at five different viewing fields. Collagen density was then determined by measuring the blue-stained area in each field of view.<sup>21</sup> Overall, measurements of epidermal thickness,

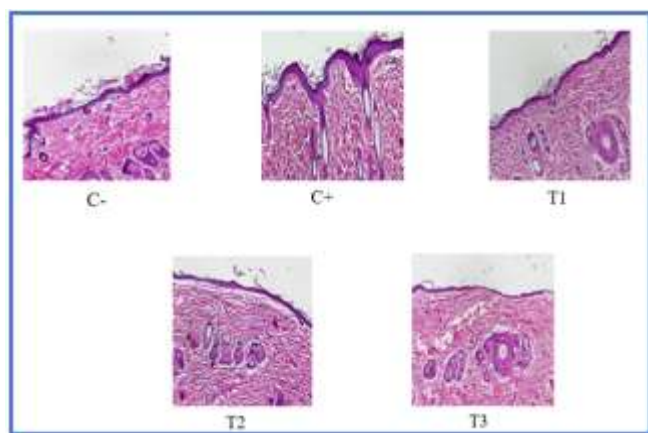
fibroblast cell count, and collagen density were performed with the aid of a light microscope.<sup>22</sup>

#### Statistical Analysis

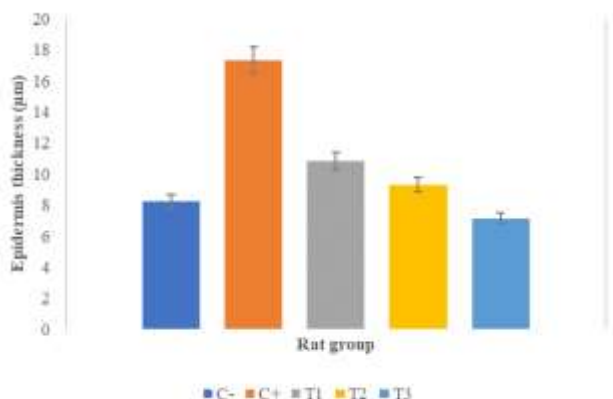
The obtained data were tested for normality and homogeneity, followed by analysis of variance (ANOVA) with a 95% significance level. If the test results showed a significant difference, the LSD (Least Significant Difference) test was performed. The data was processed using SPSS 2016.

### Results and Discussion

This study focused on evaluating the effects of *G. cowa* Roxb on the skin condition of *Rattus norvegicus* rats that had previously been exposed to UVB light, and comprehensively observed the changes that occurred in the rats' skin. The staining of the back skin of a *Rattus norvegicus* rat with HE revealed changes due to the extract treatment, particularly in mitigating the negative effects of UV rays on epidermal thickness (Figure 1). The study examined how *G. cowa* Roxb fruit rind extract inhibits the increase in skin thickness in *Rattus norvegicus* rats exposed to UVB light (Figure 2).



**Figure 1:** Histology of the thickness of the epidermis of *Rattus Norvegicus* rats. C- (Negative control group); C+ (Positive control group); T1 (Treatment group 1); T2 (Treatment group 2); T3 (Treatment group 3)



**Figure 2:** Effect of *G. cowa* Roxb fruit peel extract on histological changes in epidermis thickness in *Rattus norvegicus* rats skin exposed to UV

Histopathological analysis of epidermis thickness is shown in Figure 2. In C-, the average epidermal thickness was recorded at 8.2 µm, with normal skin structure and no signs of hypertrophy or abnormal thickening. This result indicates a healthy basic skin condition, unaffected by external stimulation. In contrast, the C+ group exhibited

drastic epidermal thickening, reaching a thickness of 17.35 µm. All other groups showed lower thickness compared to the C+ group. Specifically, the C- group showed a 52.74% reduction. For the treatment groups, T1 reduced thickness by 37.58%, T2 by 46.22%, and T3 was the most effective with a 58.73% reduction compared to C+. Thus, no group experienced an increase in epidermal thickness compared to C+; instead, all showed a decrease. Statistical analysis using the ANOVA test further confirmed the existence of a highly statistically significant difference in epidermal thickness between all groups ( $p = 0.000$ ;  $p < 0.05$ ). These results suggest that *G. cowa* Roxb. extract has remarkable potential as a therapeutic agent for conditions involving epidermal thickening. *G. cowa* Roxb. extract has shown promise as a treatment for skin conditions that involve thickening, mainly because it is rich in phenols and has strong antioxidant properties. The extract's advantages for skin health, along with its bioactive constituents, suggest a potential role in managing skin disorders. The extract's antioxidant properties and phenolic composition may help in mitigating oxidative stress and inflammation. The accumulation of ROS and inflammation leads to alterations in skin structure and function, which include histopathological changes such as erythema, increased dehydration of the skin, decreased hydration levels on the skin surface, epidermal and dermal hypertrophy, and an increase in epidermal thickness; all of these are indicators of compromised skin barrier function.<sup>23-24-25</sup> UVB radiation triggers several effects, including erythema, skin edema, hyperplasia, leukocytosis infiltration, dermal blood vessel dilation, and vascular hyperpermeability. Additionally, this irradiation causes epidermal proliferation, hardening of the skin's epidermis, and thickening of the skin.<sup>26-27</sup>

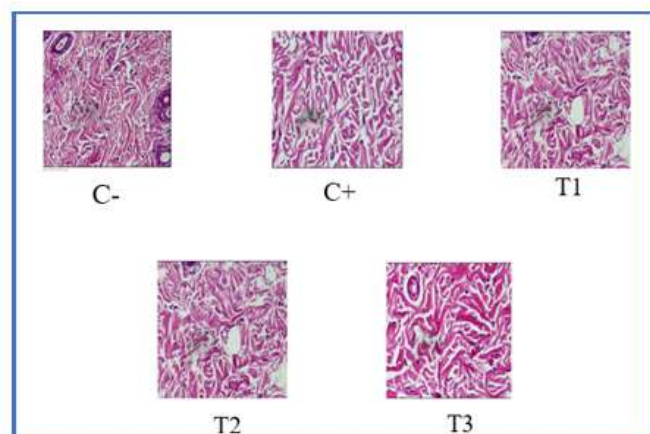
This evidence confirms that UVB light is effective in inducing epidermal thickening as a response to damage. However, the groups (T1, T2, and T3) that received *G. cowa* Roxb fruit peel extract exhibited significantly reduced epidermal thickening induced by UVB, approaching normal conditions. The thickness of the epidermis in the T1 group (3% concentration) was 10.83 µm, in the T2 group (5% concentration) was 9.33 µm, and in the T3 group (8% concentration) was 7.16 µm. Statistical analysis using the ANOVA test revealed a highly significant difference in epidermis thickness ( $p = 0.000$ ;  $p < 0.05$ ) among all groups. These data clearly show that rats exposed to UVB light significantly thicken their epidermis. The *G. cowa* Roxb fruit skin extract reduces epidermis thickening in *Rattus norvegicus* rats as its concentration rises.

The application of this extract to the skin effectively reduces the thickness of the epidermis, a typical sign of UV-B-induced photoaging. Impressively, the suppression of epidermal thickening increased with higher concentrations of *G. cowa* Roxb. This phenomenon contrasts with the typical photoaging conditions that generally occur. For example, after seven days of UVB exposure, the epidermis of the skin in group C- tended to thicken significantly, indicating a cellular proliferative response to the damage. UVB radiation is known to substantially accelerate the growth of skin cells, resulting in a 2.25-fold increase in skin thickness.<sup>28</sup> However, in the group that received the extract, there was an opposite effect, where the skin thickness was successfully reduced, and at some amounts, it approached normal levels. The relationship between the dose of *G. cowa* Roxb fruit peel extract used and the skin thickness of *Rattus norvegicus* rats is crucial. This information provides valuable guidance in selecting skin care treatments and determining the appropriate dosage for product formulation. Previous studies have also demonstrated that extracts from *Garcinia* species exhibit promising photoprotective effects, potentially enhancing skin health and treating conditions such as actinic keratosis. For example, in vitro and in vivo studies of *G. brasiliensis* extract showed its ability to protect skin from UV damage as effectively as commercial sunscreens.<sup>29</sup> The extract was shown to decrease inflammatory markers and oxidative stress in skin cells, indicating its capacity to protect against photodamage. Therefore, *G. cowa* Roxb extract could be a powerful remedy for managing skin issues such as photoaging and other problems caused by UV exposure by affecting the skin's thickness.

HE staining in histopathological analysis identifies fibroblasts. These cells appear purple and exhibit a characteristic spindle-shaped morphology, often with one or more nuclei within. Another important



characteristic is their basophilic nature, which means that they have an affinity for basic dyes such as hematoxylin (Figure 3). In this study, significant changes were observed in *Rattus norvegicus* rat dermal fibroblasts after their skin was exposed to UV-B light. UVB exposure can alter the number, shape, and function of fibroblasts, which affects how collagen and elastin—essential components for maintaining skin strength and elasticity—are produced. Examining these fibroblast alterations is crucial in comprehending the sun's damage to the skin and the potential benefits of the tested extracts. *G. cowa* Roxb. rind extract showed a significant recovery in the number of fibroblasts in the T group. While the negative control group (C-) had 22.5 fibroblast cells and the positive control group (C+) had only 13.5 cells, administration of *G. cowa* Roxb. rind extract gradually increased the number of fibroblasts. In group T1, the number of fibroblasts increased to 19.5 cells. T2 showed a further increase with 21 cells, while T3 demonstrated the most significant growth with 27.3 cells. The number of fibroblasts in the C+ group was recorded at 13.5. impressively, all other groups showed a significant increase in the number of fibroblasts compared to this value. Similarly, the treatment interventions also successfully increased the number of fibroblasts: T1 to 19.5 (44.44% higher), T2 to 21 (55.56% higher), and T3 recorded the most drastic increase to 27.3 (102.22% higher). Overall, these data indicate that both the negative control condition and all three treatments (T1, T2, and T3) were effective in substantially increasing the number of fibroblasts.



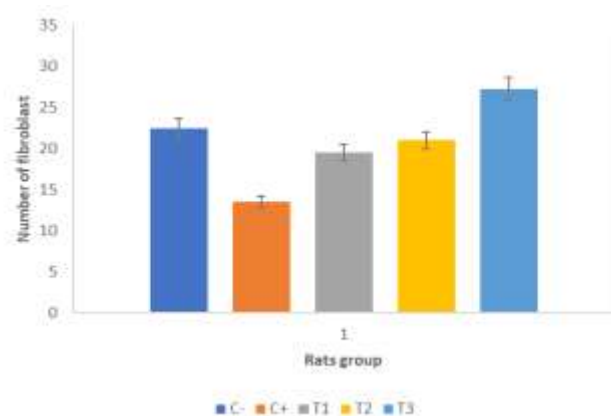
**Figure 3:** *Rattus norvegicus* rat skin fibroblast cells. C- (Negative control group); C+ (Positive control group); T1 (Treatment group 1); T2 (Treatment group 2); T3 (Treatment group 3). These cells will appear purple and have a characteristic spindle-shaped morphology

The results of the ANOVA test confirmed a highly significant difference in the number of fibroblasts between groups ( $p = 0.000$ ;  $p < 0.05$ ). This study demonstrates that the extract from *G. cowa* Roxb fruit peel alters the number of fibroblast cells in the skin of *Rattus norvegicus* rats following UVB exposure. To identify which groups showed significant differences specifically, a post hoc LSD test was conducted. The results showed a significant difference between the negative control and the positive control groups ( $p = 0.003$ ), confirming UVB-induced damage. Furthermore, there was a significant difference between the positive control and treatment groups 2 ( $p = 0.017$ ) and treatment group 3 ( $p = 0.000$ ), indicating the effectiveness of the extract at higher concentrations. Additionally, a significant difference was observed between treatment groups 1 and 3 ( $p = 0.011$ ), suggesting that the effect of increasing the number of fibroblasts is dose-dependent. UVB radiation is known to damage fibroblast cells through various pathways, ultimately leading to cellular dysfunction and even apoptosis (programmed cell death). One of the main mechanisms is increased production of ROS, DNA damage, and weakening of the cell repair system. UVB exposure also damages mitochondria, which are the primary centers of cell energy production, thereby further increasing

ROS production. This condition can accelerate cellular aging or even cell death, depending on the cell's autophagy response.<sup>29</sup> Another study indicated that short exposure to UVB laser light can rapidly kill cells by damaging the fibroblast lipid membrane, thereby reducing the cell's ability to survive.<sup>30</sup> The significant increase in ROS levels from UVB radiation causes oxidative stress and damage to cells, which is connected to essential changes in metabolism, including how glycerophospholipids and glutathione are handled.<sup>31</sup> Our findings indicate that *G. cowa* Roxb has the potential to mitigate these damaging effects, helping to maintain the viability and number of fibroblasts, which are crucial for skin health.

This study has indicated that *G. cowa* Roxb rind extract has significant potential in protecting the skin from UVB-induced damage. The extract has been shown to mitigate UVB-induced damage by increasing the viability and function of fibroblasts (T1, T2, and T3). The powerful antioxidant properties of the extract help reduce stress caused by UVB rays, as lab studies indicated that more fibroblast cells survived after being exposed to UVB.<sup>32</sup> This evidence indicates that the extract can help shield cells from UV damage, likely by lowering the amounts of ROS that rise after UVB exposure, which helps keep fibroblast cells alive and boosts their survival. *G. cowa* Roxb contains vitamin C and vitamin A at 151.4 mg/100 g extract and 1.23 mg/100 g extract, respectively, while its flavonoid content is 69.5 mg/100 g extract. The levels of vitamin C, vitamin A, and flavonoids in *G. cowa* Roxb can contribute to the development of natural bioactive substances, which can be further developed into anti-aging cosmetic products.<sup>9</sup>

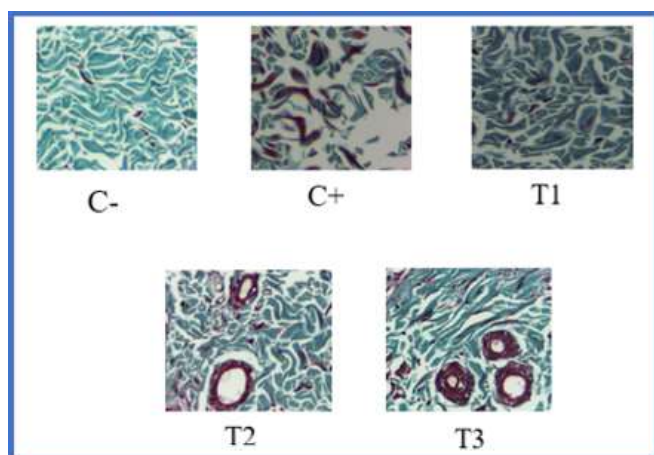
Additionally, UVB radiation is known to alter the production of collagen and fibronectin, both of which are essential for maintaining skin integrity.<sup>33</sup> Furthermore, studies in *Rattus norvegicus* rats have shown that the addition of *G. cowa* Roxb. rind extract significantly increased the strength of these rats.<sup>34</sup> Extracts that are high in bioactive compounds, particularly polyphenols, can lower oxidative stress and shield cells from UV damage by removing harmful ROS created by UVB exposure.<sup>35</sup> Various types of *Garcinia* extracts have also been shown to decrease the production of MMP-1 and oxidative stress markers, ultimately increasing the chances of cell survival.<sup>36</sup> Further lab studies indicate that extracts from *Garcinia* species can help more fibroblasts survive UVB exposure, similar to how commercial sunscreens work.<sup>37</sup> In live tests, these extracts also helped restore natural antioxidants like glutathione, which are usually reduced by UV radiation.<sup>38</sup>



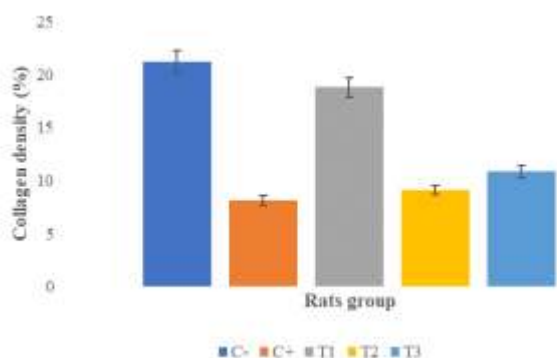
**Figure 4:** The change in the number of fibroblast cells of *Rattus norvegicus* rats exposed to UVB rays.

The flavonoid and vitamin C content in the fruit peel extract of *G. cowa* Roxb is believed to be key factors. These phytochemicals can reduce the production of ROS in human and animal skin cells, and they can also help regulate genes related to facial wrinkles. Evaluations from in vivo also revealed that these extracts can replenish endogenous antioxidants, such as glutathione, which UV radiation typically depletes.<sup>39</sup> *G. cowa* Roxb fruit peel extract contains flavonoids and vitamin C. These phytochemicals reduce the amount of ROS generated by skin fibroblasts from both human and animal models, and they also

regulate the expression of genes associated with facial wrinkles.<sup>40</sup> Since skin fibroblasts are important for making collagen and keeping the correct levels of collagen and elastin in the skin, the vitamin C in these extracts might help these cells do their important jobs.<sup>41</sup> Flavonoids are substances that help fibroblasts grow faster by boosting the production of transforming growth factor beta (TGF- $\beta$ ), which then causes fibroblast growth.<sup>42</sup> Previous studies have also shown that phenolic compounds can increase fibroblast migration and stimulate collagen production in various types of animals.<sup>43</sup> Thereby, it seems that the mix of flavonoids and vitamin C in *G. cowa* Roxb fruit peel extract is likely what helps mouse skin fibroblast cells recover and protect themselves from UVB light, suggesting it could be a favorable option for skin care. Direct exposure to UVB rays has the potential to damage skin collagen in *Rattus norvegicus* rats, a damage that will eventually result in a significant decrease in collagen density. This damage not only affects the structure of the skin but can also disrupt its vital functions (Figures 5 and 6).



**Figure 5:** Collagen density of *Rattus norvegicus* rat skin exposed to UVB radiation. C- (Negative control group); C+ (Positive control group); T1 (Treatment group 1); T2 (Treatment group 2); T3 (Treatment group 3).



**Figure 6:** Collagen density of *Rattus norvegicus* rat skin exposed to UV

Collagen density analysis showed significant differences between groups. The negative control group (C-) had an average collagen density of 21.18%, while the positive control group (C+) experienced a drastic decrease of up to 8.13%. This decrease in the C+ group indicated a significant decline of 61.6% in collagen fiber density compared to the negative control group. On the other hand, the treatment group given *G. cowa* showed indications of improvement. Collagen density in group T1 was 18.79%; subsequently, it was 9.11% in T2 and then increased to 10.83% in T3 (Figure 6). Specifically, *G. cowa* Roxb fruit skin extract at a concentration of 3% showed the ability to maximally stop the decrease in collagen density percentage. Statistical analysis using

the ANOVA test revealed a significant difference in collagen density percentage between groups ( $p = 0.000$ ;  $p < 0.05$ ). This significant difference was seen between the C+ group and the C- and T1 groups ( $p$ -value  $< 0.05$ ). between groups T1 and T2 ( $p$ -value = 0.008), as well as between groups T1 and T3 ( $p$ -value = 0.04). These results demonstrate that administering an extract of *G. cowa* Roxb to *Rattus norvegicus* rats exposed to UVB radiation can effectively prevent damage to collagen. Collagen damage due to UVB rays is not only structural but also functional, which ultimately accelerates the skin aging process (Figure 6).

Further research indicates that UVB radiation triggers collagen degradation and reduces its production, both of which are essential for maintaining overall skin elasticity and health. UVB rays also cause oxidative stress, resulting in the buildup of ROS that damages the extracellular matrix and increases inflammation.<sup>44</sup> Researchers have found that UVB radiation increases the production of matrix metalloproteinases (MMPs), enzymes that break down collagen, while simultaneously decreasing the production of collagen. This phenomenon is where *G. cowa* Roxb. rind extract comes into play. This extract is rich in antioxidants that can inhibit UV-induced MMP activation in skin fibroblasts, thereby preventing collagen breakdown. Research has shown that antioxidants, such as astaxanthin, and vitamins C and E, when combined with collagen peptides, help reduce oxidative stress and inflammation, two key factors that contribute to sun-induced skin aging.<sup>45-46</sup> These antioxidants have been shown to reduce MMP expression, thereby maintaining collagen integrity. Recent studies also support the notion that plant extracts containing antioxidants can prevent collagen breakdown by inhibiting skin fibroblasts from producing MMPs triggered by UV light.<sup>47-48</sup> In addition to antioxidants, bioactive plant compounds such as phytochemicals and vitamins play an essential role in the production and prevention of collagen degradation. Previous studies have revealed that *G. cowa* Roxb fruit peel extract contains flavonoids and vitamin C.<sup>9</sup> Vitamin C, in particular, is an essential cofactor for collagen hydroxylase (proline and lysine) in the skin. Since collagen contains a high amount of proline and lysine, adding hydroxyl groups to both amino acids helps create new collagen, highlighting the beneficial effects of this extract on maintaining healthy and youthful skin.<sup>49</sup>

This study examined the effects of *G. cowa* Roxb extract on *Rattus norvegicus* rat skin after five weeks of treatment following exposure to UVB light. At a dose of 3%, collagen levels rose significantly compared to the C+ group. Several natural extracts have been shown to protect against UV-induced skin damage, supporting their potential to maintain high collagen levels and slow the aging process. An ethanol extract of purple cabbage prevented Wistar rats exposed to UV-B radiation from breaking down collagen.<sup>50-51</sup> The findings of this study suggest that it may help slow the loss of collagen density. The photoprotective and antioxidant properties of the extract may help maintain collagen, a key component for healthy skin. The antioxidant qualities of this extract may alleviate oxidative stress, a significant contributor to collagen breakdown. Although there is not much research on *G. cowa* Roxb., studies on related species have shown that extracts from *Garcinia* species can increase collagen production, similar to how other fruit extracts increase the expression of type I collagen.<sup>52</sup>

## Conclusion

Administration of *G. cowa* Roxb fruit peel extract inhibits the increase in skin thickness. It prevents a significant decrease in fibroblast and collagen cells in the skin of mice exposed to UV light. Histopathological analysis revealed that the epidermal thickness increased dramatically in the positive control group. In contrast, the extracts cause a reduction of the epidermal thickness in the treatment groups. The administration of *G. cowa* Roxb. fruit peel extract inhibited the increase in skin thickness and prevented a significant decrease in fibroblast cells and collagen levels in the skin of mice that were exposed to UVB rays. *G. cowa* Roxb. fruit peel extract (8%) exhibited the best inhibitory and protective effect on the skin of mice exposed to UVB rays. This study suggests that extracts from the rind of *G. cowa* Roxb are a promising natural option for reducing UVB-induced skin damage, aiding in skin repair, and slowing down skin aging, and can be incorporated into cosmetic products. This finding could pave the way

for safer, natural skin care products that tap into the potential of plant-based remedies to counteract the long-term effects of sun exposure. Future research on *G. cowa* Roxb. bark extract should focus on elucidating its photoprotective mechanisms against UVB damage and conducting long-term studies to assess safety and potential side effects.

### Conflict of Interest

The author's declare no conflict of interest.

### Author's Declaration

The authors certify that the work contained in this article is original and that they will assume responsibility for any claims related to their content.

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