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Protective effect of young coconut water on MMP-1 levels and collagen fiber thickness in male Wistar rats exposed to UVB light exposure

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ABSTRACT

Background: Exposure to ultraviolet B (UVB) light plays a key role in photoaging by increasing oxidative stress, activating inflammatory pathways, and increasing the expression of matrix metalloproteinase-1 (MMP-1), which causes dermal collagen degradation. Young coconut water contains various bioactive compounds with antioxidant activity that can protect the skin from UVB-induced damage.

Aim: This study aimed to analyze the effect of the oral administration of young coconut water on MMP-1 levels and collagen fiber thickness in male Wistar rats exposed to UVB light.

Methods: A purely experimental study with a post-test only control group design was conducted on 24 male Wistar rats divided into four groups: healthy controls (K1), UVB exposure (K2), UVB + young coconut water 4 ml/200 g BW/day (K3), and UVB + young coconut water 8 ml/200 g BW/day (K4). UVB exposure was administered for 15 days at a cumulative dose of 800 mJ/cm². MMP-1 levels were measured using an enzyme-linked immunosorbent assay method, while collagen fiber thickness was analyzed using Masson's Trichrome staining.

Results: UVB exposure significantly increased MMP-1 levels and decreased collagen fiber thickness ($p = 0.001$). Oral administration of young coconut water significantly reduced MMP-1 levels and increased collagen fiber thickness compared with the untreated UVB group, with the most optimal effect at a dose of 4 ml/200 g body weight/day.

Conclusion: Young coconut water has potential as a natural antioxidant agent that can inhibit the increase in MMP-1 and maintain collagen fiber thickness in the skin caused by UVB exposure, thus potentially being used in the prevention of photoaging.

Keywords: Young coconut water, UVB, MMP-1, Collagen, Photoaging.

Introduction

Exposure to ultraviolet radiation, particularly ultraviolet B (UVB) rays with a wavelength of 290–320 nm, is a major environmental factor contributing to skin

damage (Al-Sadek and Yusuf, 2024). UVB rays have high photon energy that can penetrate the epidermis to the upper dermis, triggering various biological changes in skin tissue (Gromkowska-Kępa *et al.*,

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2021). UVB exposure causes cellular DNA damage, increased reactive oxygen species (ROS) formation, and activation of oxidative stress and inflammatory pathways (Wei *et al.*, 2024). In contrast to UVA exposure, which is more cumulative and chronic, these effects contribute to acute photoaging, characterized by decreased skin elasticity, wrinkles, and extracellular matrix degradation (Brar *et al.*, 2025).

Physiologically, the body has a natural defense system against free radicals in the form of endogenous antioxidants that neutralize ROS so that they do not damage cellular components such as membrane lipids, proteins, and DNA (Chandimali *et al.*, 2025). The main endogenous antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which synergistically convert superoxide radicals into hydrogen peroxide and then into non-toxic water (Manful *et al.*, 2025). However, excessive UVB exposure can significantly increase ROS production, overwhelming the endogenous antioxidant system's capacity (Tran *et al.*, 2023). This imbalance is known as oxidative stress and triggers the activation of the Mitogen-Activated Protein Kinase (MAPK) and Nuclear Factor Kappa B (NF- κ B) pathways (Guo *et al.*, 2023).

MAPK and NF- κ B activation play a crucial role in increasing the expression of matrix metalloproteinase-1 (MMP-1), the primary proteolytic enzyme responsible for the degradation of collagen types I and III in the dermis (Feng *et al.*, 2024). Elevated MMP-1 levels decrease the number and thickness of collagen fibers, resulting in brittle skin structure, reduced elasticity, and wrinkle development (Bar and Valiukevičienė, 2025). This condition not only impacts esthetics but also increases the risk of precancerous lesions and skin cancer (Hussein *et al.*, 2025). Chronic exposure to UVB rays is associated with an increased incidence of non-melanoma skin cancers worldwide and decreases skin immune function (immunosuppression) (Anwaar *et al.*, 2025).

Efforts to prevent and improve skin aging are generally carried out through the use of topical cosmetics containing chemicals such as retinol or tretinoin (Milosheska and Roškar, 2022). Although effective, the use of these ingredients is often associated with side effects such as skin irritation, peeling, and increased sensitivity to sunlight (Karwal and Mukovozov, 2023). Therefore, the search for alternatives based on natural ingredients that are safe, readily available, and possess antioxidant activity is becoming increasingly important, especially in tropical countries like Indonesia, which are rich in biological resources.

Young coconut water, the endosperm fluid from the *Cocos nucifera* L. fruit, is a potential natural ingredient (Zhang *et al.*, 2024). Young coconut water, particularly from the green wulung coconut variety, is known to contain various bioactive compounds, such as vitamin C, L-arginine, L-methionine, cytokinins, selenium, and

microminerals, such as zinc (Zn), manganese (Mn), and copper (Cu) (Shi *et al.*, 2025). These compounds have antioxidant and anti-inflammatory activity by increasing the activity of the enzymes SOD, CAT, and GPx and reducing malondialdehyde (MDA) levels, a marker of lipid peroxidation (Zulaikhah *et al.*, 2022). Furthermore, vitamin C and amino acids in young coconut water play an important role in collagen synthesis by stimulating the expression of collagen-forming genes and increasing the activity of hydroxylase enzymes, which are necessary for the formation of a strong and stable collagen structure (Boo, 2022).

Although various benefits of young coconut water have been reported, research specifically examining the effect of oral administration of young coconut water on MMP-1 levels and collagen fiber thickness in skin damaged by UVB exposure remains limited. Therefore, this study aimed to analyze the effect of oral administration of young coconut water on MMP-1 levels and collagen fiber thickness in male Wistar rats exposed to UVB light. The results provide a scientific basis for the use of young coconut water as a natural antioxidant agent in preventing photoaging.

Materials and Methods

Place and time of the research

This study was conducted at the Integrated Biomedical Laboratories of the Faculty of Medicine, Universitas Islam Sultan Agung (UNISSULA), and the CITO Laboratory. In October 2025, the study lasted for 30 days, covering adaptation, treatment, sampling, and laboratory analysis.

Design and type of research

This was a purely experimental study with a post-test only control group design. This study aimed to analyze the effect of oral administration of young coconut water on MMP-1 levels and collagen fiber thickness in male Wistar rats exposed to UVB light.

Experimental animals

The experimental animals used were 24 male Wistar rats (*Rattus norvegicus*), approximately 2 months old and weighing 150–200 g, obtained from Semarang City. Rats were housed in standard cages with good ventilation, controlled room temperature, and a 12-hours light–dark cycle. They were fed a standard AD-II diet and drinking water *ad libitum* (du Sert *et al.*, 2020).

All rats underwent a 7-day adaptation period to acclimate to the laboratory environment before treatment.

Group division and treatment

After the adaptation period, the mice were randomly divided (simple random sampling) into four groups, each consisting of 6 mice, namely:

- K1 (healthy control): mice were not exposed to UVB light but were subjected to the same handling and

sham oral gavage procedure (without any solution administration) to ensure equal procedural stress across groups. They received standard food and drinking water ad libitum.

- K2 (negative control): mice were exposed to UVB light and given water orally using a probe.
- K3 (low-dose treatment): Mice were exposed to UVB light and orally administered young coconut water at a dose of 4 ml/200 g BW/day.
- K4 (high dose treatment): mice were exposed to UVB light and given young coconut water at a dose of 8 ml/200 g BW/day, divided into two administrations (morning and afternoon).

All treatments were administered for 15 consecutive days.

Giving young coconuts water

Young coconut water was obtained from 5–7-month-old green wulung coconuts (*Cocos nucifera* var. *viridis*). Coconut water was collected daily from freshly opened coconuts to maintain nutritional stability. Orally administered using a tube according to the dosage for each treatment group for 15 days (Shi *et al.*, 2025).

Based on published literature, young coconut water contains bioactive compounds such as vitamin C (approximately 2.4–5.0 mg/100 ml), L-arginine (approximately 30–50 mg/100 ml), L-methionine in trace amounts, cytokinins, and essential micronutrients including potassium, zinc, manganese, copper, and selenium (Zulaikhah, 2019; Zhang *et al.*, 2024; Shi *et al.*, 2025).

No additional laboratory analysis was performed to quantify the exact biochemical composition of the coconut water batch used in the present study. Coconuts from the same cultivar (green wulung) and similar maturity stage (5–7 months) were used to minimize compositional variability.

UVB exposure

Groups K2, K3, and K4 were exposed to UVB using a 20-watt UVB TL lamp with a wavelength of 312 nm and an irradiation distance of 20 cm. The exposure dose was 1 minimal erythema dose (MED) or 100 mJ/cm² for 10 minutes, carried out every 2 days on days 1, 3, 5, 7, 9, 11, 13, and 15, so that the total cumulative dose reached 800 mJ/cm² (Jin *et al.*, 2010).

On non-irradiation days (days 2, 4, 6, 8, 10, 12, and 14), the animals were maintained under standard housing conditions without UVB exposure and handled similarly to irradiation days to minimize handling bias. No sham exposure procedure was performed. The UVB dose was calculated based on the lamp output specifications, irradiation distance (20 cm), and exposure duration, resulting in a cumulative dose of 800 mJ/cm² over the treatment period.

Sampling

On day 16 (24 hours after the last UVB exposure), all mice were anesthetized intraperitoneally using 100 mg/kg BW of ketamine until euthanasia was

achieved. Approximately 0.5 ml of blood was drawn through the orbital sinus to determine MMP-1 levels. Subsequently, dorsal skin tissue was cleaned of hair and taken in sections measuring approximately 1 × 1 cm² for histological analysis (Li *et al.*, 2025).

Examination of MMP-1 level

Blood samples were centrifuged for 15 min to obtain serum. MMP-1 levels were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Elabscience). The results are expressed in pg/mL (Aydin *et al.*, 2025).

Check the thickness of the collagen fibers

Skin tissue was fixed in 10% neutral buffered formalin for 24–48 hours and then processed through dehydration, clearing, infiltration, paraffin embedding, and sectioning using a microtome at a thickness of 4–5 µm. The slides were stained using Masson's Trichrome to visualize collagen (Baidoo *et al.*, 2023).

The thickness of the collagen fiber was measured using a light microscope at 400× magnification, calibrated using an ocular and stage micrometer. Five random fields of view were selected from each dermis slide, and the average collagen fiber thickness was expressed in µm (Woessner *et al.*, 2019).

Statistical analysis

Statistical analysis was performed using SPSS software version 29 (IBM Corp., Armonk, NY). Data were first tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test.

If data were normally distributed and homogeneous, comparisons among groups were analyzed using one-way analysis of variance followed by the least significant difference post hoc test. If the assumptions of normality were not met, the non-parametric Kruskal–Wallis test was used, followed by the Mann–Whitney *U* test for pairwise comparisons. Data are presented as mean ± SD. A *p*-value < 0.05 was considered statistically significant.

Ethical approval

This research was approved by the Medical/Health Research Bioethics Commission, Faculty of Medicine, Universitas Islam Sultan Agung (Certificate No. 598/XI/2025/Komisi Bioetik).

Results

This study evaluated the effect of the oral administration of young coconut water on MMP-1 levels and collagen fiber thickness in male Wistar rats exposed to UVB light. Twenty-four rats were divided into four groups (K1–K4), each consisting of six rats. Table 1 presents the mean MMP-1 levels and collagen fiber thickness in each group.

The results showed that the group of rats exposed only to UVB light (K2) had the highest mean MMP-1 levels, at (6.615 ± 0.162 pg/ml). Meanwhile, the lowest mean MMP-1 levels were found in the treatment group receiving young coconut water at a dose of 4 ml/200 g body weight/day (K3), at 4.435 ± 0.278 pg/ml. The

Table 1. Mean MMP-1 levels and collagen fiber thickness in each group.

Variables	K1 (Control)	K2 (UVB)	K3 (UVB + 4 mL AKM)	K4 (UVB + 8 mL AKM)	p-value
MMP-1 (pg/mL)	4.926 ± 0.072	6.615 ± 0.162	4.435 ± 0.278	4.955 ± 0.028	0.001*
Collagen (µm)	27.700 ± 5.370	18.100 ± 4.684	35.866 ± 6.549	30.700 ± 8.295	0.001*

Note: Kruskal–Wallis test for MMP-1; one-way analysis of variance for collagen thickness. AKM: young coconut water.

Table 2. Comparison of MMP-1 Levels between Groups (Mann–Whitney U test).

Groups	K1	K2	K3	K4
K1	–	0.004*	0.054	0.872
K2		–	0.004*	0.004*
K3			–	0.044*
K4				–

Note: Significant at $p < 0.05$.

healthy control group (K1) and the 8 ml/200 g BW/day treatment group (K4) each showed mean MMP-1 levels of 4.926 ± 0.072 pg/ml and 4.955 ± 0.028 pg/ml, respectively.

The Shapiro–Wilk normality test showed that MMP-1 levels in groups K1 and K4 were normally distributed ($p > 0.05$), whereas those in groups K2 and K3 were not normally distributed ($p < 0.05$). A homogeneity of the variance test using Levene’s test showed data homogeneity ($p = 0.081$). Therefore, the analysis was continued using the nonparametric Kruskal–Wallis test. The Kruskal–Wallis test revealed a significant difference in MMP-1 levels between the four groups ($p = 0.001$). A follow-up Mann–Whitney test revealed significant differences between K1 and K2, K2 and K3, K2 and K4, and between K3 and K4 ($p < 0.05$). However, no significant differences were found between K1 and K3 or between K1 and K4 ($p > 0.05$). Table 2 presents a summary of the comparisons between groups.

The results of the collagen fiber thickness analysis showed that the K3 treatment group had the highest mean collagen fiber thickness, at 35.866 ± 6.549 µm, followed by the K4 group (30.700 ± 8.295 µm), healthy control group K1 (27.700 ± 5.370 µm), and untreated UVB-exposed group K2, which showed the lowest mean thickness (18.100 ± 4.684 µm).

Representative Masson’s Trichrome-stained skin sections from each experimental group (K1–K4) are presented in Figure 1. Consistent with the quantitative findings, the UVB-only group (K2) exhibited visibly reduced collagen density and disrupted fiber organization, whereas the treatment groups, particularly K3, exhibited thicker and more densely arranged collagen fibers.

The Shapiro–Wilk normality test showed that all groups had a normal distribution of data ($p > 0.05$). The homogeneity of the variance test using Levene’s test showed data homogeneity ($p = 0.402$). Based on

these results, one-way ANOVA was used for statistical analysis.

The results of one-way ANOVA showed a statistically significant difference between the four groups ($p = 0.001$). Further post hoc LSD tests revealed significant differences between K1 and K2, K1 and K3, K2 and K3, and K2 and K4 ($p < 0.05$). However, no significant differences were found between K1 and K4 or between K3 and K4 ($p > 0.05$). Table 3 presents the results of the intergroup comparison.

Discussion

The results of this study indicate that UVB exposure significantly increased MMP-1 levels and decreased collagen fiber thickness in the skin of male Wistar rats. Conversely, the oral administration of young coconut water reduced MMP-1 levels and increased collagen fiber thickness in the treatment group, with the most optimal effect observed at a dose of 4 ml/200 g body weight/day. These findings confirm the role of UVB light as a primary trigger of photoaging and the potential of young coconut water as a natural photoprotective agent.

The increase in MMP-1 levels in the UVB-only group (K2) is consistent with the widely reported pathophysiological mechanisms of photoaging (Moon *et al.*, 2008). UVB radiation triggers the formation of large amounts of ROS in skin tissue, which exceeds the capacity of endogenous antioxidant systems such as SOD, CAT, and GPx (Dunaway *et al.*, 2018). ROS accumulation acts as a second messenger that activates intracellular signaling pathways, particularly mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) (Gunaseelan *et al.*, 2017). Activation of this pathway increases the expression of the transcription factor Activator Protein-1 (AP-1), which plays a direct role in the induction of MMP-1 gene transcription (Kim *et al.*, 2018). MMP-1 is the primary collagenase responsible for the degradation of collagen

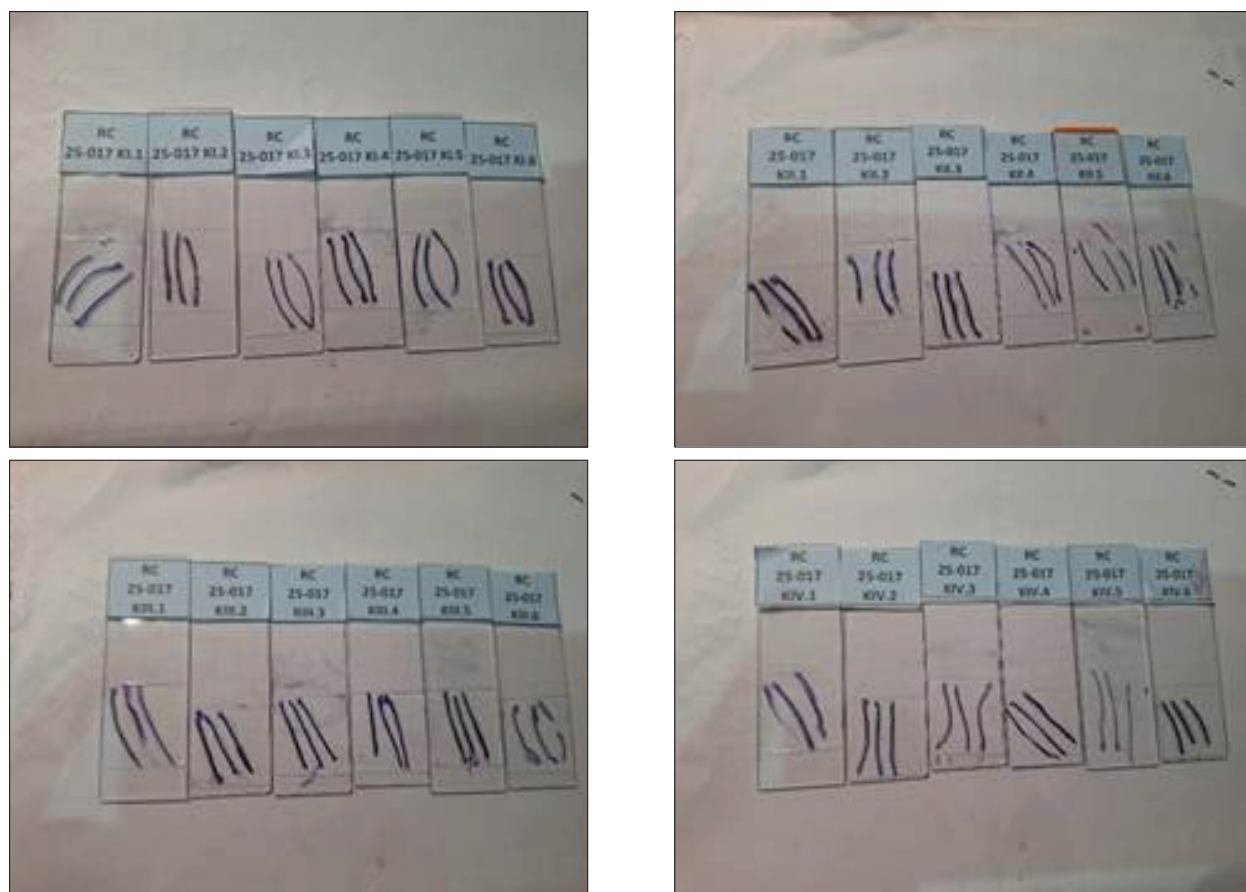


Fig. 1. Representative dermal sections stained with Masson's Trichrome from the experimental groups (K1–K4).

Table 3. Comparison of collagen fiber thickness between groups (post hoc LSD).

Groups	K1	K2	K3	K4
K1	–	0.017*	0.038*	0.425
K2		–	0.000*	0.003*
K3			–	0.176
K4				–

Note: Significant at $p < 0.05$.

types I and III, so its increased levels contribute to decreased collagen fiber thickness and weakened dermal structure (Brennan *et al.*, 2003).

In addition to increasing collagen degradation, UVB exposure also inhibits new collagen synthesis by suppressing the Transforming Growth Factor- β (TGF- β)/Smad pathway (Geng *et al.*, 2021). AP-1 activation due to oxidative stress is known to decrease the expression of the TGF- β receptor type II (T β RII) and increase the expression of Smad7, which functions as an inhibitor of Smad2/3 phosphorylation (He *et al.*, 2014). Consequently, the transcription of collagen-constituting genes, such as COL1A1 and COL3A1, is significantly reduced. This mechanism explains why

group K2 had the highest MMP-1 levels and the lowest collagen fiber thickness compared to the other groups (Zhu *et al.*, 2025).

Oral administration of young coconut water to groups K3 and K4 suppressed the increase in MMP-1 levels caused by UVB exposure. This effect is thought to be related to the bioactive compounds in young coconut water, particularly vitamin C, L-arginine, L-methionine, cytokinins, selenium, and trace minerals such as Zn, Mn, and Cu (Zulaikhah, 2019). Vitamin C is a powerful antioxidant that plays a role in neutralizing ROS by donating electrons, thereby reducing oxidative stress and inhibiting the activation of the MAPK and NF- κ B pathways (Oudemans-van Straaten *et al.*, 2014).

By reducing AP-1 and NF- κ B activation, MMP-1 expression can be suppressed, as reflected in the lower MMP-1 levels in the treated group compared to the untreated UVB group (Lee *et al.*, 2024).

In addition to its antioxidant role, vitamin C is also an essential cofactor for the prolyl and lysyl hydroxylase enzymes, which are involved in the proline and lysine hydroxylation stages of collagen synthesis (Alberts *et al.*, 2025). This process is essential for the formation of a strong and stable triple helix collagen structure. Therefore, the increased collagen fiber thickness in the K3 and K4 groups reflects not only reduced collagen degradation but also increased new collagen synthesis (Singh *et al.*, 2023).

L-arginine in young coconut water also contributes to the protective effects observed. This amino acid is a precursor of nitric oxide (NO), which can increase the activity of endogenous antioxidant enzymes such as SOD and GPx at physiological concentrations (Wu *et al.*, 2021). ROS accumulation can be suppressed by increasing endogenous antioxidant capacity, thereby reducing the activation of the MAPK–AP-1 and NF- κ B pathways (Behl *et al.*, 2021). Furthermore, L-arginine stimulates the TGF- β pathway, which plays a role in regulating collagen synthesis by dermal fibroblasts (Hamanaka *et al.*, 2025). These findings are consistent with those of previous studies that reported increased skin elasticity and the number of collagen and elastin fibers after L-arginine supplementation (Miler *et al.*, 2024).

The L-methionine and selenium contained in young coconut water also support antioxidant mechanisms by increasing GSH synthesis and GPx activity (Li *et al.*, 2024). GSH is a major intracellular antioxidant that plays a crucial role in detoxifying reactive oxygen species (ROS) and lipid peroxides (Dawi *et al.*, 2024). Reducing oxidative stress can suppress inflammatory pathway activation and MMP-1 expression (Lee *et al.*, 2024). Micronutrients such as Zn, Mn, and Cu function as cofactors for antioxidant enzymes (Cu/Zn-SOD and Mn-SOD), thereby strengthening the overall antioxidant defense system of the skin (Darenskaya *et al.*, 2025).

Interestingly, the results of this study demonstrated that a dose of 4 mL/200 g BW/day (K3) produced a more pronounced protective effect than 8 mL/200 g BW/day (K4), both in reducing MMP-1 levels and increasing collagen fiber thickness. This indicates a biphasic dose–response relationship rather than a simple linear correlation between dose and biological effect. Such responses are consistent with the concept of hormesis, in which low-to-moderate doses of bioactive compounds exert beneficial adaptive effects, whereas higher doses may attenuate or even counteract these effects (Jodynis-Liebert and Kujawska, 2020).

At moderate concentrations, the antioxidant components of young coconut water, particularly vitamin C, amino acids, and trace minerals, may

effectively enhance endogenous antioxidant defense systems, suppress ROS-mediated MAPK/AP-1 signaling activation, and reduce MMP-1 expression. However, several biological mechanisms may limit this protective effect at higher doses. First, the antioxidant pathways may become functionally saturated, reducing the additional benefit despite increased intake. Second, cellular transport systems, such as sodium-dependent vitamin C transporters (SVCTs), exhibit saturation kinetics, potentially limiting the intracellular accumulation of ascorbic acid at higher concentrations. As a result, increasing the administered dose may not proportionally increase cellular antioxidant capacity.

Moreover, high concentrations of certain antioxidants, including vitamin C, exert mild pro-oxidant effects under specific conditions, particularly in the presence of transition metals, potentially leading to redox imbalance rather than protection (Peper, 2009). Excessive antioxidant supplementation may also trigger feedback regulatory mechanisms that downregulate endogenous antioxidant enzyme activity, thereby diminishing overall redox homeostasis. Collectively, these mechanisms may explain why the higher dose did not provide superior protection compared with the moderate dose observed in this study.

This study demonstrates that young coconut water has potential as a natural anti-photoaging agent through mechanisms such as reducing oxidative stress, inhibiting the MAPK and NF- κ B pathways, suppressing MMP-1 expression, and increasing dermal collagen synthesis and thickness. However, this study has several limitations that should be acknowledged. First, MMP-1 levels were measured only in serum using an enzyme-linked immunosorbent assay, which may not fully reflect local dermal MMP-1 expression or activity within skin tissue. Previous studies have shown that UVB-induced MMP-1 expression occurs predominantly in dermal fibroblasts and keratinocytes at the tissue level (Brennan *et al.*, 2003; Choi *et al.*, 2020). Therefore, the absence of immunohistochemical (IHC) analysis limits our ability to directly evaluate the localization and intensity of MMP-1 expression within the dermis.

Second, not only MMP-1 but also its endogenous inhibitors, particularly TIMP-1, regulate extracellular matrix turnover. The balance between MMPs and TIMPs plays a critical role in collagen homeostasis (Cabral-Pacheco *et al.*, 2020). The lack of TIMP-1 measurement in this study prevents a comprehensive assessment of collagen degradation–inhibition dynamics.

Third, this study did not quantitatively analyze the composition of young coconut water. Natural products such as coconut water exhibit variability in bioactive compounds depending on cultivar, maturity stage, and environmental factors (Zhang *et al.*, 2024; Shi *et al.*, 2025). Therefore, the absence of compositional

standardization may limit reproducibility and comparability with other studies.

Fourth, the healthy control group (K1) did not undergo sham gavage procedures, which may introduce potential procedural stress bias compared with treatment groups receiving repeated oral administration. Repeated handling and gavage reportedly influence physiological stress responses in rodents (du Sert *et al.*, 2020; Li *et al.*, 2025).

Finally, the relatively short UVB exposure duration represents an acute photodamage model and may not fully mimic chronic photoaging conditions. Chronic UV exposure induces cumulative structural and molecular alterations in dermal collagen (Gromkowska-Kępką *et al.*, 2021; Feng *et al.*, 2024). To strengthen the mechanistic understanding, future studies should include longer exposure periods, tissue-level molecular analyses, and broader oxidative stress and inflammatory markers. Previous veterinary studies in Indonesia have also emphasized the importance of molecular and morphological investigations to better understand animal health problems and support the development of effective disease prevention strategies in livestock and experimental animal models (Kurniawan *et al.*, 2025).

Conclusion

Exposure to UVB light significantly increases MMP-1 levels and decreases collagen fiber thickness in the skin of male Wistar rats. Oral administration of young coconut water can suppress the increase in MMP-1 and significantly increase collagen fiber thickness, with the most optimal effect at a dose of 4 ml/200 g body weight per day. These findings indicate that young coconut water has the potential to be a natural antioxidant agent in preventing skin damage due to UVB exposure and photoaging processes, although further research is still needed to strengthen its clinical application.

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Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

MA, PS, and STZ: Conceived, designed, and coordinated the study. ARK and MHE: designed data collection tools, supervised field sample and data collection, and performed laboratory work and data entry. EPH and BB: Validation, supervision, and formal analysis of data. DAP and SR: Contributed reagents, materials, and analysis tools. MA, AG, and BPP: Carried out the statistical analysis and interpretation and participated in manuscript preparation. All authors have read, reviewed, and approved the final version of the manuscript.

Data availability

All data are available in the revised manuscript.

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