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Original Article

Protective effect of blemish balm cream containing flesh of Salak Pondoh *(Salacca zalacca* [Gaert.] Voss) fruit extract against ultraviolet B-induced photoaging

Wahyu Widowati[a], Dani Dani[a], Vera Vera[a], Fadhilah Haifa Zahiroh[b]*

[a]Faculty of Medicine, Maranatha Christian University, Bandung, West Java, Indonesia, [b]Aretha Medika Utama, Bimolecular and Biomedical Research Center, Bandung, West Java, Indonesia

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Abstract

Objectives: Ultraviolet (UV) light, particularly ultraviolet B (UVB), is a major external factor that accelerates skin aging by generating reactive oxygen species (ROS), which damage skin cells and biological molecules. The flesh of snake fruit (*Salacca zalacca*) contains compounds that may help neutralize ROS and protect the skin from premature aging. This research investigates the protective effects of a Blemish Balm (BB) cream formulated with *S. zalacca* fruit extract against UVB-induced photoaging. **Materials and Methods:** *S. zalacca* flesh extract (SFE) was formulated with various ingredients, resulting in the BB cream containing SFE (BBSFE). Antioxidant and antiaging *in vitro* assays were measured by H_2O_2 scavenging, ferric reducing antioxidant power (FRAP), collagenase, and tyrosinase inhibition activities on SFE and BBSFE. Male mice (*Mus musculus L.*) were used as a UVB-induced photoaging model by shaving the dorsal skin and exposing it to UVB irradiation twice daily for 14 days, except for the negative control. The treated mice were smeared with BBSFE once and twice daily for 14 days. Hematoxylin-eosin staining is used to detect collagen fiber damage and inflammation. An immunohistochemistry (IHC) assay was used to analyze the p53, MMP-2, MMP-13, and COL4A1 protein expression. Quantitative real-time reverse transcription polymerase chain reaction assay was used to analyze the transforming growth factor-β1 (*Tgf-β1*), interleukin *(Il)10*, and *Il18* gene expression. **Results:** BBSFE shows strong antioxidant and antiaging effects by inhibiting FRAP, H_2O_2, collagenase, and tyrosinase. It enhances collagen levels, reduces skin inflammation, downregulates p53, MMP-2, MMP-13, and *Il18*, and upregulates COL4A1, *Tgf-β1*, and *Il10* in mice skin. **Conclusion:** BBSFE has potential as an antiaging agent *in vitro* and *in vivo* studies.

Keywords: *Antiaging, Antioxidant, Blemish balm cream, Photoaging, Salacca zalacca (Gaert.) Voss*

Introduction

Aging is a physiological process characterized by functional decline and structural changes in cells and tissues. Skin aging is the most visible form due to the skin's position as the outermost organ. Among external factors, ultraviolet (UV) exposure is the most significant contributor to skin aging [1]. UV rays penetrate the skin and generate reactive oxygen species (ROS), which are highly reactive and damaging. Histologically, ROS effects are visible in the dermis through collagen fiber degradation [2]. Collagen, a key component of the extracellular matrix (ECM), contributes to skin strength and elasticity. ROS can upregulate collagenase activity, leading to collagen degradation and wrinkle formation [3]. UV exposure also stimulates melanin production, causing hyperpigmentation through tyrosinase activity, the key enzyme in melanogenesis [4].

ROS further activate signaling pathways that promote inflammation, skin damage, aging, and even cancer [5]. Key affected molecules include p53, matrix metalloproteinases (MMPs), transforming growth factor-β1 (TGF-β1), and interleukins (ILs). p53 mediates

***Address for correspondence:** Dr. Wahyu Widowati, Faculty of Medicine, Maranatha Christian University, Jl. Prof. drg. Surya Sumantri, M.P.H. No. 65, Bandung 40164, West Java, Indonesia.
E-mail: wahyu_w60@yahoo.com

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keratinocyte apoptosis following mitochondrial DNA damage [6]. UV also stimulates MMP synthesis via TNF-α release by keratinocytes and fibroblasts, contributing to inflammation and aging [7]. MMPs include stromelysins (MMP-3, -10, and -11), gelatinases (MMP-2 and -9), and collagenases (MMP-1, -8, -13) [8], and can suppress collagen-producing proteins like COL4A1 [9]. ROS also activate MAPK signaling (p38, JNK, and ERK), increasing AP-1 expression, which inhibits TGF-β1 regulation and alters skin structure [10]. In addition, ROS stimulates IL-18 expression [11] while reducing IL-10 expression [12].

Blemish balm cream is a multifunctional cosmetic product providing moisturization and UV protection [13,14]. Consumers increasingly demand natural antiaging products due to safety and environmental concerns [15,16]. The flesh of *Salacca zalacca* (snake fruit) cultivar Pondoh is rich in phenolics and flavonoids, with superior antioxidant capacity compared to other cultivars [17]. Extracts from its peel show antiaging activity [18], yet the antioxidant potential of flesh extract in BB cream formulations remains unexplored. This study evaluates blemish balm cream containing *S. zalacca* flesh extract (BBSFE) through *in vitro* assays (H_2O_2 scavenging, ferric reducing antioxidant power [FRAP], collagenase, and tyrosinase inhibition) and *in vivo* molecular analysis targeting p53, MMP-2, MMP-13, COL4A1, *Tgf-β1*, *Il10*, and *Il18*.

Materials and methods

Ethical approval

This study was approved by the Ethical Committee of iRATco Veterinary Laboratory Services, Bogor, Indonesia (No: 4.2.003-1/KEHI/IX/2022).

Preparation of blemish balm cream containing *Salacca zalacca* flesh extract

Salacca zalacca fruits were collected in Bogor, West Java, Indonesia and identified by the Biology Department, Bandung Institute of Technology. The fruits were cleaned, deseeded, dried, and ground into powder, then extracted with 70% ethanol. The extract was filtered and evaporated to form a paste, followed by lactose addition [19]. *S. zalacca* flesh extract (SFE) was processed under GMP by PT FAST (Depok, Indonesia) (CoA No. Batch 001.11.23.ERTL.01) [20]. The BB cream formulation (BBSFE) was produced by CV SKINSOL, West Java, Indonesia, by incorporating SFE into a base BB cream (BBBC) consisting of standard cosmetic ingredients. BBBC served as the vehicle control, whereas 3W Clinic sunscreen (EAN 8809192574892) was used as a comparator [19].

Ferric reducing antioxidant power assay

The antioxidant capacity was evaluated using a modified FRAP protocol [21]. Sample solutions (0–1000 μg/mL) of SFE, BBSFE, and BBBC were mixed with FRAP reagent (TPTZ and ferric chloride hexahydrate) and incubated for 30 min at 37°C. Absorbance was measured at 595 nm using a microplate reader.

H_2O_2 scavenging assay

Samples (60 μL) were mixed with 5 mM H_2O_2 (3 μL) and 1 mM ferrous ammonium sulfate (12 μL), then incubated at room temperature. After adding 1 mM 1,10-phenanthroline (75 μL), absorbance was read at 510 nm [22,23].

Collagenase inhibitory assay

Collagenase activity was measured using a modified method [24,25]. Samples (7.81–250 μg/mL) were mixed with collagenase enzyme, Tricine buffer, and FALGPA substrate, incubated at 37°C for 20 min, and read at 335 nm.

Tyrosinase inhibitory assay

Samples (3.13–100 μg/mL) were incubated with fungal tyrosinase enzyme and phosphate buffer (pH 6.8) at room temperature. After L-DOPA substrate addition, absorbance was measured at 470 nm [25,26].

Animal experimental design

Male DDY mice (10–11 weeks, 30–35 g) were housed under controlled conditions with food and water *ad libitum*. Animals were divided into six groups (n = 4): (I) negative control, (II) UVB-induced control, (III) vehicle control (BBBC), (IV–V) BBSFE-treated (once or twice daily), and (VI) comparator (3W Clinic sunscreen). Prior to treatment, mice were shaved and UVB-induced for 14 days (twice daily), followed by cream application for 14 days [27]. BBSFE cream was applied topically in a thin layer, and the mice were monitored to minimize removal due to grooming or licking, ensuring consistent exposure.

Skin collection and termination

On day 15, mice were sacrificed via cervical dislocation. Skin samples were collected, fixed in 10% formalin for histology and immunohistochemistry (IHC), and stored in 0.9% NaCl at –80°C for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) [28].

Histopathological assay

Skin samples were processed through dehydration, xylene clearing, and paraffin embedding. Sections (5 μm) were stained with hematoxylin-eosin and analyzed using light microscopy and ImageJ for collagen and inflammation quantification [29].

Immunohistochemistry

Paraffin-embedded sections (5 μm) underwent antigen retrieval, peroxidase blocking, and primary antibody incubation with anti-p53, COL4A1, MMP-2, and MMP-13 (Elabsci) overnight. Detection was conducted using biotinylated secondary antibodies and DAB chromogen. ImageJ software was used for analysis [20].

qRT-PCR analysis of *Tgf-β1, Il10* and *Il8* gene expression

RNA was extracted using Tri Reagent and purified with Direct-zol RNA MiniPrep. cDNA was synthesized using iScript Reverse Transcription Supermix, and qRT-PCR was conducted using SSOFAST EvaGreen Supermix on a real-time PCR system. Gene expression of *Tgf-β1*, *Il10*, and *Il18* was normalized to *β-actin* [30].

Statistical analysis

Data were tested for normality. Parametric data were analyzed with analysis of variance followed by Tukey or Dunnett's T3 *post hoc* tests. Nonparametric data were analyzed using Kruskal–Wallis and Mann–Whitney *U* tests. Significance was set at $P < 0.05$ [31].

Results

Antioxidant and antiaging activity of blemish balm cream containing *Salacca zalacca* flesh extract

The antioxidant capacity of SFE, BBSFE, and BBBC was assessed using FRAP and H_2O_2 scavenging assays [Figure 1a and b and Supplementary Table 1]. At the highest concentration, BBSFE showed the strongest FRAP reducing capacity (84.62%) and H_2O_2 scavenging activity (IC_{50} = 98.73 μg/mL), compared to SFE (FRAP 64.92%, IC_{50} = 139.67 μg/mL) and BBBC (FRAP 7.63%, IC_{50} = 2812.77 μg/mL). These results suggest BBSFE possesses superior antioxidant activity.

The antiaging potential was evaluated through collagenase and tyrosinase inhibitory assays [Figure 1c and d]. BBSFE demonstrated the strongest collagenase inhibition (IC_{50} = 70.93 μg/mL), outperforming SFE (113.52 μg/mL) and BBBC (361.90 μg/mL). Tyrosinase inhibition was also highest in BBSFE (IC_{50} =36.15 μg/mL), followed by SFE (43.74 μg/mL) and BBBC (157.56 μg/mL). These findings highlight the superior antiaging activity of BBSFE compared to SFE and BBBC.

Effect of blemish balm cream containing *Salacca zalacca* flesh extract on skin histopathology in aging mice model

UVB induction led to reduced collagen proportion and mild inflammation in mouse skin, characterized by diminished collagen fibers and immune cell infiltration [Figure 2a]. BBSFE treatment twice daily (BBSFEII) significantly increased collagen proportion (64.32%) compared to the positive control (45.30%), nearly matching the sunscreen comparator (65.92%) [Figure 2b]. Inflammation scores showed mild inflammation (score 1) in the positive group. BBSFEII reduced inflammation to score 0.5, slightly higher than the sunscreen group (score 0.25), indicating a moderate anti-inflammatory effect [Figure 2c].

Effect of blemish balm cream containing *Salacca zalacca* flesh extract on p53, MMP-2, MMP-13, and COL4A1 expression of skin in aging model mice

Immunohistochemistry (IHC) staining revealed that UVB exposure increased p53 expression in the epidermis, as evidenced by dense brown cytoplasmic staining [Figure 3a]. BBSFEII reduced p53 expression (1.24%) compared to the positive control (1.96%) and approached the level seen in the sunscreen group (0.76%) [Figure 3b], suggesting its potential to attenuate DNA damage signaling.

MMP-2 expression was elevated in UVB-induced mice (16.36%). BBSFEII significantly reduced MMP-2 levels (12.39%), even lower than the sunscreen comparator (12.89%) [Figure 3c and d]. Similarly, MMP-13 expression decreased with BBSFEII treatment (0.68% versus

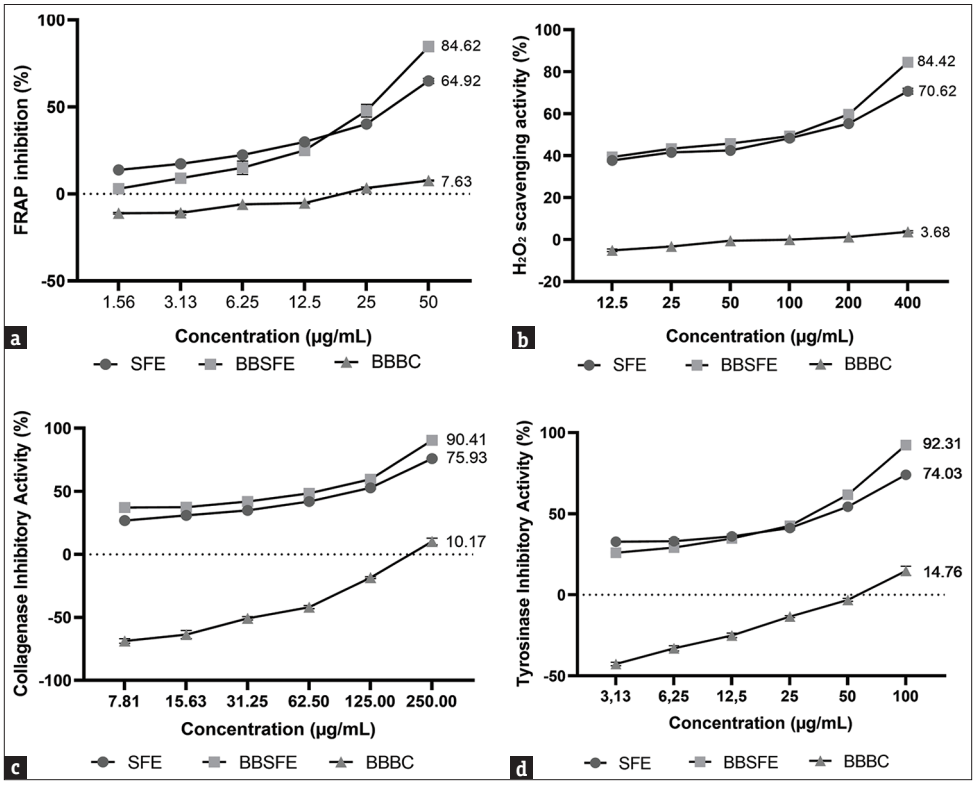

Figure 1: Effect of various concentrations of *Salacca zalacca* flesh extract (SFE), blemish balm cream containing SFE (BBSFE), and base blemish balm cream (BBBC) on (a) ferric reducing antioxidant power (FRAP) activity, (b) H_2O_2 scavenging activity, (c) collagenase inhibitory activity, and (d) tyrosinase inhibitory activity. SFE, BBSFE, and BBBC were diluted in DMSO to reach the final concentrations. All experiments were conducted in triplicate

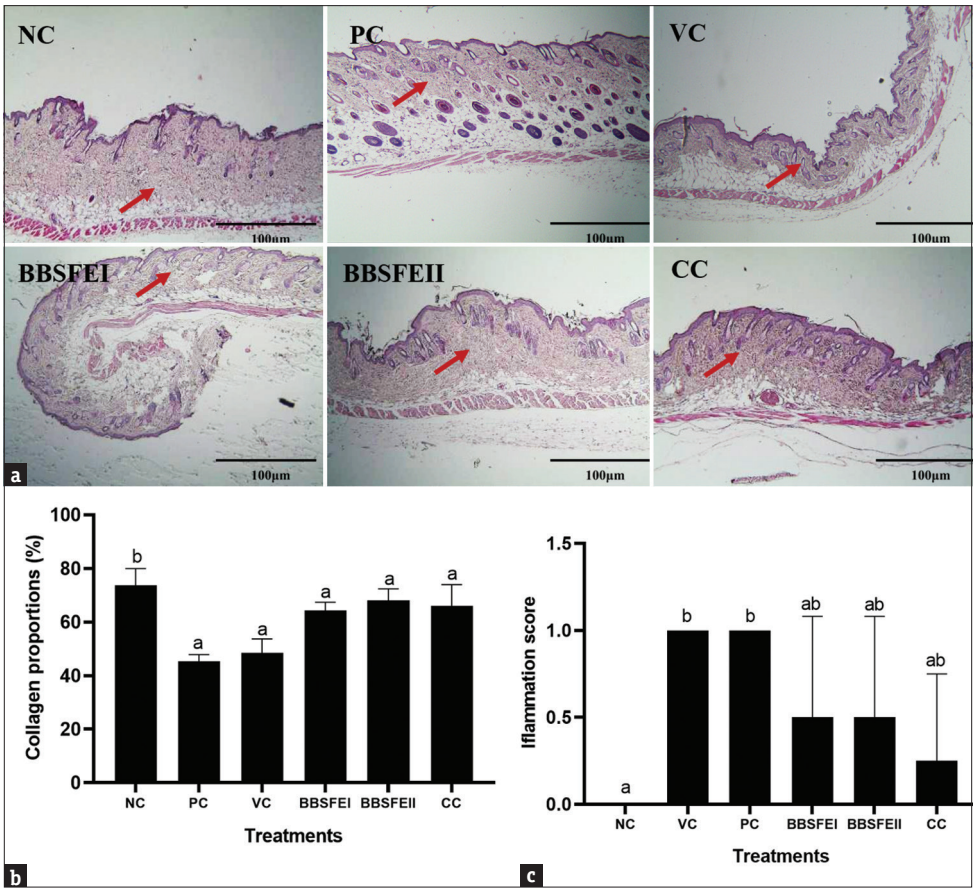

Figure 2: Effects of blemish balm cream containing SFE on skin histopathology in aging mice model. (a) Skin histopathology, (b) Collagen proportion, (c) Inflammation score. *Data are presented as the mean and standard deviation over four replications. NC: Negative control, PC: Positive control, VC: Vehicle control (BBBC), BBSFEI: treatment with BBSFE 1x topically, BBSFEII: treatment with BBSFE 2x topically, CC: Comparator control (sunscreen cream). The differences in letters (a and b) for collagen proportion (%) show a significant difference between the treatment based on Tukey's HSD *post hoc* test ($P < 0.05$) test. According to the Mann–Whitney *U* test ($P < 0.05$), the differences in the letters (a, ab, and b) for the inflammation score indicated significant differences between treatments. Letters are assigned according to statistical grouping from the lowest to highest values and not according to the identity of the control group

the positive control (0.96%) and was slightly lower than the sunscreen group (0.73%) [Figure 3e and f], indicating inhibition of ECM degradation. COL4A1, a collagen-synthesizing protein, was significantly upregulated by BBSFEII (13.17%) compared to the positive control (7.04%), with values close to the sunscreen group (12.66%) [Figure 3g and h]. This suggests enhanced skin structural protein synthesis by BBSFE.

Effect of BBSFE on *Tgf-β1, Il10,* and *Il18* gene expression in aging mice model

Gene expression analysis showed that BBSFEII significantly upregulated *Tgf-β1* (0.79) and *Il10* (0.83) mRNA levels compared to the positive control (0.51 and 0.45, respectively), approaching those of the sunscreen comparator (0.85 for *Il10*) [Figure 4a and b]. These results suggest that BBSFE promotes antiaging and anti-inflammatory gene expression. Conversely, BBSFEII significantly downregulated pro-inflammatory *Il18* expression (2.29) compared to the UVB group (6.23) [Figure 4c], indicating reduced inflammation at the molecular level. Overall, these results suggest that BBSFE modulates gene expression associated with inflammation and aging, providing both protective and reparative effects in UVB-induced skin aging.

Discussion

Premature skin aging is mainly driven by ultraviolet B (UVB) exposure, which generates excessive ROS. These ROS act as signaling molecules that activate upstream pathways such as MAPK (ERK, JNK, p38) and NF-κB. The activation of these pathways leads to increased expression of p53, MMPs, and pro-inflammatory cytokines such as IL-18, while simultaneously suppressing protective regulators including COL4A1, TGF-β1, and IL-10. The imbalance between these pro-aging and anti-aging mediators results in collagen degradation, elastin loss, inflammation, apoptosis, and accumulation of senescent cells, all of which contribute to the visible signs of photoaging [32].

Managing skin aging, especially in sun-exposed areas like the face, is critical as it reflects age-related physiological changes. BB cream has emerged as a multifunctional topical product serving both cosmetic and antiaging purposes [14]. Incorporating *S. zalacca* fruit flesh extract into BB cream offers promising potential due to its phytochemicals – polyphenols, tannins, flavonoids, and monoterpenoids – which act as antioxidants and antiaging agents by protecting skin from oxidative and photodamage [2]. Supporting this, LC-MS/MS

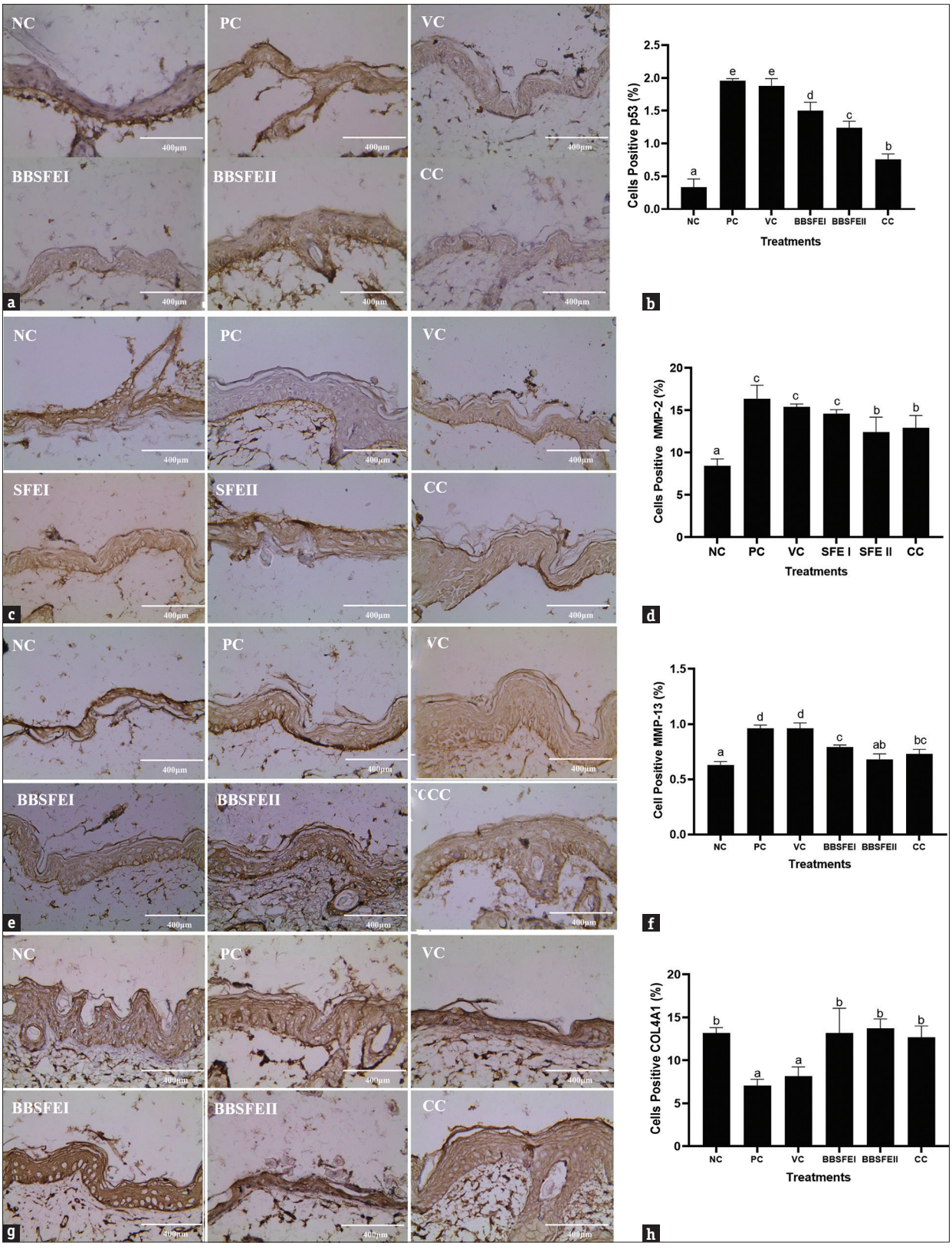

Figure 3: Effect of blemish balm cream containing SFE on p53, MMP-2, MMP-13, COL4A1 protein expression in aging mice model. (a) Skin histopathology of p53 protein expression, (b) p53 protein expression, (c) Skin histopathology of MMP-2 protein expression, (d) MMP-2 protein expression, (e) Skin histopathology of MMP-13 protein expression, (f) MMP-13 protein expression, (g) Skin histopathology of COL4A1 protein expression, (h) COL4A1 protein expression. *Data are presented as the mean and standard deviation over four replications. NC: Negative control, PC: Positive control, VC: Vehicle control (BBBC); BBSFEI: treatment with BBSFE 1 × topically, BBSFEII: Treatment with BBSFE 2 × topically, CC: Comparator control (sunscreen cream). The differences in letters indicated significant differences between treatments based on the Tukey HSD *post hoc* test ($P < 0.05$). Groups sharing the same letter are not significantly different, whereas groups with different letters differ significantly ($P < 0.05$). Letters are assigned according to statistical grouping from the lowest to the highest values, and not according to the identity of the control group

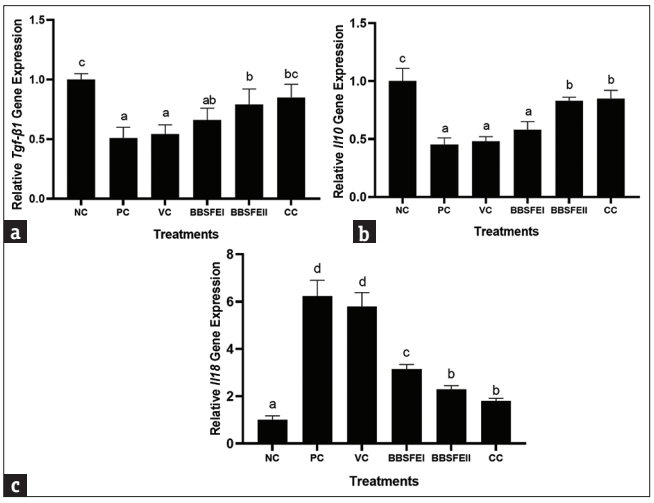

Figure 4: Effect of blemish balm cream containing SFE on (a) *Tgf-β1*, (b) *Il10*, and (c) *Il18* gene expression in aging mice model. *Data are represented as the mean and standard deviation over four replications. NC: Negative control, PC: Positive control, VC: Vehicle control (BBBC), BBSFEI: Treatment with BBSFE 1x topically, BBSFEII: Treatment with BBSFE 2x topically, CC: comparator control (sunscreen cream). Different letters above the bars indicate significant differences between groups according to Tukey's HSD *post hoc* test ($P < 0.05$). Groups sharing the same letter are not significantly different, whereas groups with different letters differ significantly ($P < 0.05$). Letters are assigned according to statistical grouping from the lowest to the highest values, and not according to the identity of the control group

analysis of *S. zalacca* extract reported in our previous study demonstrated the presence of chlorogenic acid, epicatechin, naringenin, and apigenin as major constituents [25]. These compounds are known to possess strong antioxidant and antiaging properties, further reinforcing the potential of BBSFE in topical formulations [33].

This study demonstrates that BBSFE possesses strong antioxidant activity, evident from its H_2O_2 scavenging and FRAP reducing capacity. This is attributed to its flavonoid content, known to neutralize ROS through hydrogen donation and free radical scavenging [22]. The flavonoids in BBSFE also show potent FRAP reducing capacity, falling into the high antioxidant category with IC_{50} values <50 μg/mL [34]. This activity stems from the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} by donating electrons [35]. Supporting this, our previous study in UV-induced human fibroblast cells as photoaging cell model demonstrated that *S. zalacca* extract upregulated the antioxidant gene GPX-1 and reduced the oxidative DNA damage marker 8-OHdG, while also lowering apoptosis, confirming its cellular antioxidant potential [33]. The relevance of these assays can be explained as follows: the H_2O_2 scavenging activity of BBSFE, which reflects direct ROS neutralization [22], is consistent with our previous findings showing increased GPX-1 expression in UV-induced fibroblasts [33]. Similarly, the strong reducing capacity demonstrated by the FRAP assay [34,35] aligns with the observed reduction of 8-OHdG levels [33], since enhanced electron-donating potential can limit oxidative DNA damage. Together, these findings indicate that the antioxidant effects of BBSFE observed in chemical assays are mechanistically connected to its protective role at the cellular level.

BBSFE also exhibits significant antiaging potential through inhibition of collagenase and tyrosinase activities. Its collagenase inhibition may be influenced by flavonols, which have shown strong inhibitory effects on collagen-degrading enzymes [36]. By suppressing collagenase, BBSFE helps preserve ECM integrity and prevent wrinkle formation. The tyrosinase inhibition is likely due to the flavonoid structure, which is effective in blocking melanogenesis [37], thereby reducing hyperpigmentation associated with photoaging. BBSFE's superior performance compared to SFE and BBBC may be due to the presence of additional active agents such as glutathione, titanium dioxide, and niacinamide, which synergistically enhance its antioxidant and antiaging activity. Collectively, these findings suggest that BBSFE interferes with key enzymatic processes, including collagen degradation and melanogenesis, thereby protecting against ROS-induced premature aging, hyperpigmentation, and structural skin damage.

Histological assessment in a UVB-induced aging mouse model shows that UVB exposure leads to collagen degradation and dermal inflammation [38]. Mechanistically, these effects are mediated by ROS-induced activation of Nuclear Factor Kappa B (NF-κB) signaling, which upregulates inflammatory cytokines and MMPs, thereby driving ECM breakdown. BBSFE application – especially twice daily – significantly preserves collagen structure and reduces inflammation. This protective effect is likely due to the extract's polyphenol content, which neutralizes ROS and suppresses NF-κB-mediated inflammatory responses [39,40]. *S. zalacca* extract has also been shown to neutralize H_2O_2 and inhibit collagenase and elastase activity, contributing to ECM stability and collagen maintenance [33].

ROS can induce p53 overexpression in the epidermis, a key factor in photoaging that triggers apoptosis and skin thinning [41,42]. In this study, p53 levels increased significantly in the UVB-exposed control group. BBSFE application markedly reduced p53 expression, possibly due to its antioxidant constituents such as polyphenols and flavonoids, which can counter oxidative stress and modulate apoptotic signaling [43]. Beyond its role as a marker of oxidative stress, p53 acts as a transcriptional regulator of downstream effectors, which mediate cell cycle arrest and apoptosis [44]. By attenuating ROS-induced p53 activation, BBSFE may prevent excessive keratinocyte apoptosis and the accumulation of senescent cells, thereby preserving epidermal structure and delaying photoaging.

UV-induced ROS also activates MMPs via NF-κB and AP-1 signaling, particularly MMP-2, which degrades type IV collagen and contributes to wrinkle formation [45,46]. ROS is known to activate upstream signaling cascades, particularly MAPK (ERK, JNK, and p38) and NF-κB, which subsequently induce AP-1 activity and MMP expression, leading to collagen breakdown [47]. The observed reduction of MMP-2 by BBSFE suggests that the extract may indirectly modulate these pathways, thereby preventing ECM degradation. Elevated MMP-2 expression was observed in UVB-exposed mice. BBSFE application effectively reduced MMP-2 levels,

comparable to or slightly lower than the sunscreen comparator. The reduction in MMP-2 is likely due to the polyphenolic content of the extract, which is known to inhibit MMP activity and support wound healing [48,49].

Similarly, MMP-13, which degrades type II collagen and contributes to ECM breakdown and skin carcinogenesis, was overexpressed in the UVB-exposed group [50,51]. MMP-13 expression is largely regulated by ROS-sensitive pathways, including MAPK and NF-κB/AP-1 signaling, which promote transcription of collagen-degrading enzymes. By suppressing oxidative stress, BBSFE may attenuate the activation of these upstream cascades, thereby limiting MMP-13 transcriptional upregulation. This mechanism not only prevents ECM breakdown but may also reduce the risk of UV-induced skin carcinogenesis associated with chronic MMP-13 overexpression. BBSFE treatment significantly downregulated MMP-13, possibly due to flavonoids that neutralize ROO• and NO• radicals, thus preventing MMP overactivation [52]. Taken together, these findings suggest that BBSFE preserves dermal structure by modulating ROS-driven MAPK/NF-κB signaling and suppressing MMP-mediated collagen degradation.

COL4A1, an essential component for collagen synthesis and basement membrane integrity, is often suppressed under UV stress through MMP-mediated degradation. UVB exposure reduced COL4A1 expression, whereas BBSFE application significantly restored its levels, comparable to sunscreen. Flavonoids in *S. zalacca* likely contribute to this effect by both inhibiting MMPs and upregulating COL4A1 gene expression, as supported by other *in vitro* findings [53,54]. This restoration of COL4A1 suggests that BBSFE not only prevents collagen degradation but also supports membrane stability, thereby maintaining dermal–epidermal integrity under UV stress.

UV-induced ROS also inhibits TGF-β1 expression, a key regulator of skin regeneration and collagen production [10]. In this study, the BBSFE application reversed UV-induced suppression of *Tgf-β1*. This effect may be mediated by the presence of polyphenols in the extract, which have been shown to activate the TGF-β1 pathway in aged fibroblasts and enhance ECM protein synthesis [55,56]. By restoring TGF-β1 signaling, BBSFE helps sustain collagen transcription and promote ECM remodeling, thereby counteracting one of the major mechanisms of UVB-induced photoaging.

IL-10 is a key anti-inflammatory cytokine that suppresses pro-inflammatory mediators and helps maintain immune homeostasis in the skin [57]. UVB exposure reduced IL-10 expression in the UVB-exposed control group. BBSFE significantly restored *Il10* levels, which can be attributed to anti-inflammatory phytochemicals in *S. zalacca*, including chlorogenic acid [58,59]. Chlorogenic acid has been shown to increase IL-10 expression, thereby helping mitigate ROS-induced inflammation [60]. Increased IL-10 may further inhibit NF-κB activation, thereby reducing downstream expression of pro-inflammatory cytokines and protecting against UVB-induced cutaneous inflammation.

In contrast, IL-18 is a pro-inflammatory cytokine associated with skin damage and premature aging due to its role in activating NF-κB and IFN-γ pathways [61,62]. UV exposure significantly upregulated IL-18 expression, while BBSFE application reversed this effect. The flavonoid content in BBSFE likely contributes to *Il18* suppression by modulating cytokine pathways and reducing ROS levels [63,64]. By downregulating IL-18, BBSFE may attenuate NF-κB-mediated transcription of pro-inflammatory mediators, thereby limiting chronic inflammation and protecting skin structure during UV-induced photoaging.

UVB-induced ROS plays a central role in photoaging by causing oxidative damage at multiple cellular levels. ROS triggers mitochondrial DNA damage and nuclear DNA fragmentation, leading to oxidative stress and p53 activation, which subsequently promote apoptosis and epidermal thinning. ROS also induce lipid peroxidation, which exacerbates cellular stress. In parallel, ROS activates NF-κB signaling, resulting in increased IL-18 and decreased IL-10 expression, thereby amplifying inflammation. Moreover, ROS stimulates AP-1 activity, leading to MMP-2 and MMP-13 overexpression, degradation of COL4A1, and subsequent collagen loss. Collectively, these events culminate in ECM breakdown, inflammation, apoptosis, and premature skin aging. BBSFE counteracts these processes by directly scavenging ROS, inhibiting oxidative stress, and modulating inflammatory and collagen-degrading pathways, thereby preserving dermal structure and delaying photoaging [Figure 5].

The limitation of this study is the absence of an *in vivo* group treated with SFE alone. Although the BB base was included as a vehicle control to account for formulation effects, the lack of an SFE-only group makes it difficult to fully distinguish the extract's specific role. Another limitation relates to the animal experimental design. Although mice were monitored to minimize removal of the cream due to grooming or licking, the actual degree of percutaneous absorption was not measured, and cream reduction cannot be completely excluded. Moreover, the RNA preservation method used may have affected sample integrity and contributed to variability in the expression of TGF-β1, IL-10, and IL-18, as tissues were promptly processed after collection and stored only briefly in 0.9% NaCl at − 80°C before RNA extraction, yet the lack of a cryoprotectant buffer could still pose a risk of partial RNA degradation. In addition, upstream signaling pathways such as MAPK (ERK, JNK, p38) and NF-κB, which are known to be involved in UVB-induced photoaging, were not specifically investigated. Future studies should incorporate an SFE-only treatment group, direct skin permeation assays, formulation optimization, or protective measures such as occlusive application and collars to validate dermal delivery, exclude potential confounding factors, optimized RNA preservation techniques using cryoprotectant-based solutions to ensure more reliable transcript stability, and analyze these signaling cascades to provide more definitive mechanistic insights into the anti-photoaging effects of BBSFE.

Conclusion

BBSFE has an antioxidant and antiaging activity by *in vitro* assay. Applying BBSFE on the mice skin twice daily can increase the proportion of collagen and minimize inflammation in the mice skin. In addition, BBSFE can also reduce the

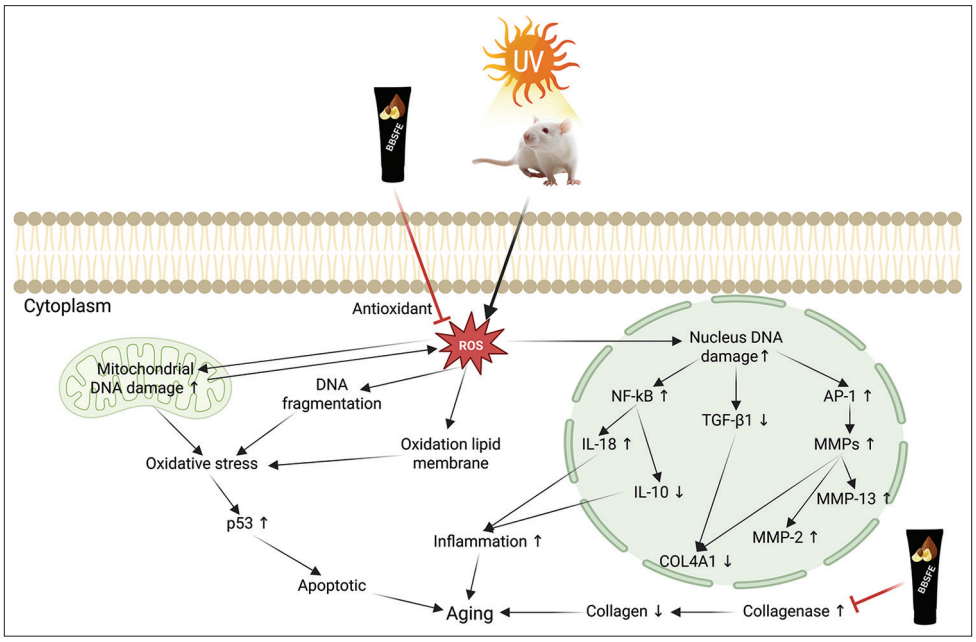

Figure 5: Proposed mechanisms of blemish balm cream containing *S. zalacca* flesh extract (BBSFE) as antiaging potential. UVB-induced ROS drive DNA damage, oxidative stress, inflammation, apoptosis, and collagen degradation, culminating in premature skin aging (black arrows). BBSFE counteracts these processes by scavenging ROS, reducing oxidative stress and inflammation, and restoring collagen-related proteins and cytokines (red arrows)

p53, MMP-2, MMP-13, and *Il18* expression and increase the expression of COL4A1, *Tgf-β1*, and *Il10*. BBSFE has the potential as an antiaging product by providing protection to the face from premature aging due to UV exposure.

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Data availability statement

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

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Supplementary material

Supplementary Table 1: The IC_{50} value of ferric reducing antioxidant power activity, H_2O_2 scavenging, collagenase inhibition, and tyrosinase inhibition activity of *Salacca zalacca* flesh extract, Blemish Balm Cream containing *Salacca zalacca* flesh extract, dan base Blemish Balm cream

Assays	Sample	Equation	*R*2	IC_{50} (µg/mL)
FRAP	SFE	y=1.0188x +14.667	0.99	34.68
activity	BBSFE	y=1.6537x +35.997	0.99	28.06
	BBBC	y=0.3944x −10.185	0.91	152.60
H_2O_2	SFE	y=0.0814x +38.631	0.99	139.67
scavenging	BBSFE	y=0.1116x +38.982	0.99	98.73
	BBBC	y=0.0189x −3.1613	0.79	2812.77
Collagenase	SFE	y=0.1954x +27.819	0.99	113.52
inhibition	BBSFE	y=0.2198x + 34.409	0.99	70.93
	BBBC	y=0.3176x −64.939	0.97	361.90
Tyrosinase	SFE	y=0.4373x + 30.871	0.99	43.74
inhibition	BBSFE	y=0.6817x + 25.356	0.99	36.15
	BBBC	y=0.5378x −34.738	0.91	157.56

BBBC: Base Blemish Balm cream, SFE: *Salacca zalacca* flesh extract, BBSFE: Blemish Balm Cream containing SFE, FRAP: Ferric reducing antioxidant power, IC_{50}: Half maximal inhibitory concentration