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Original Article

COMPARATIVE *IN VITRO* ANTI-INFLAMMATORY, WOUND-HEALING, ENZYME-INHIBITORY, AND ANTIMICROBIAL ACTIVITIES OF *BOSWELLIA DALZIELII* LEAF AND STEM BARK AQUEOUS EXTRACTS

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ABSTRACT

Boswellia dalzielii (Burseraceae) (*B. dalzielii*) is traditionally used in West African communities to treat wounds, inflammatory skin disorders, mental disorders, and microbial infections, yet its dermatological potential has not been fully validated scientifically. This study evaluated the comparative *in vitro* cytocompatibility, wound-healing, anti-inflammatory, enzyme-inhibitory, and antibacterial activities of aqueous leaf (BDL) and stem bark (BDSB) extracts. Both extracts maintained high viability and normal morphology of HaCaT keratinocytes across tested concentrations (25–200 µg/mL) and enhanced cell migration in an *in vitro* scratch wound assay, with BDSB showing the greatest effect. Extracts significantly reduced TNF-α/IFN-γ-induced production of nitric oxide, IL-6 and IL-8 as well as inhibited collagenase, elastase, and hyaluronidase activities, indicating protection of extracellular matrix components. Antibacterial assessment revealed strong activity against *Staphylococcus aureus* (MIC = 0.31 mg/mL), whereas *Pseudomonas aeruginosa* was inhibited only at higher concentrations (10 mg/mL). Importantly, this represents the first comparative evaluation of *B. dalzielii* leaf and stem bark extracts across multiple skin-relevant *in vitro* activities, scientifically supporting its folkloric usage.

KEYWORDS: *Boswellia dalzielii*; keratinocytes; wound repair; skin inflammation; antimicrobial activity; matrix-degrading enzymes; traditional medicine.

1. Introduction

The skin is the largest organ of the human body. It serves as a critical physical and immunological barrier protecting the body from environmental insults, microbial invasion, and excessive water loss [1]. The maintenance of skin integrity depends on complex biological processes including keratinocyte proliferation and migration, extracellular matrix remodeling, immune response regulation, and microbial defense mechanisms [2]. Disruption of these processes often results in impaired wound healing, chronic inflammation, and increased susceptibility to skin infections, which represent a major global health challenge [2].

Skin and wound infections are frequently associated with opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are among the most common microorganisms implicated in burn infections, chronic ulcers, and post-surgical wound complications [3]. The emergence of multidrug-resistant strains of these pathogens has intensified the search for alternative and complementary therapeutic agents [4]. Medicinal plants remain valuable sources of bioactive compounds with multifunctional pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities [5]. Their complex phytochemical composition allows them to act on multiple biological targets simultaneously, making them promising candidates for dermatological applications [5].

Inflammation is a key factor in the pathogenesis of various skin disorders and delayed wound healing [6]. Keratinocytes play an essential role in regulating cutaneous inflammatory responses by producing pro-inflammatory mediators such as nitric oxide (NO), interleukin-6 (IL-6), and interleukin-8 (IL-8) upon stimulation by cytokines including tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) [7]. Although controlled inflammatory responses are necessary for host defense and tissue repair, excessive or prolonged production of these mediators can lead to tissue damage and chronic inflammatory skin conditions [7]. Therefore, modulation of inflammatory signaling pathways in keratinocytes represents an important therapeutic strategy for managing inflammatory skin diseases.

Extracellular matrix degradation is another critical factor influencing skin aging, tissue remodeling, and wound healing [8]. Enzymes such as collagenase, elastase, and hyaluronidase are responsible for the breakdown of collagen, elastin, and hyaluronic acid, respectively, which are essential structural components that maintain skin elasticity, hydration, and mechanical strength [8]. Overexpression or dysregulation of these enzymes contributes to accelerated tissue degeneration and impaired wound repair. Consequently, inhibition of these enzymes has been widely explored as a strategy for preserving skin structure and promoting tissue regeneration.

Medicinal plants remain important sources of therapeutic agents for the management of skin-related disorders, particularly in regions where traditional medicine plays a central healthcare role [9]. The use of plant-derived products for treating wounds, infections, and inflammatory skin diseases has been widely documented in ethnopharmacological literature. *Boswellia dalzielii* is traditionally used in several West African communities for treating wounds, inflammatory conditions, and microbial infections [10, 11, 12, 13].

Boswellia dalzielii Hutch., belonging to the family Burseraceae, is extensively distributed throughout West African regions. The plant is known by several indigenous names, including Ararrabi, Basamu, and Hano in Nigeria; Kumdagneogo, Tree Man, and Volta in Burkina Faso; Piangwogu in Ghana; and Etan in Ethiopia [10]. Traditionally, various parts of the plant are utilized in folk medicine across several African countries, including Nigeria, Cameroon, Burkina Faso, Benin, and Guinea [10]. Different plant parts, particularly the leaves and bark, are prepared as decoctions and administered either topically or orally for the management of numerous health conditions [10]. Ethnomedicinal reports indicate that *B. dalzielii* has been widely used for treating gastrointestinal disorders, leprosy, septic wounds, skin infections, inflammatory diseases, rheumatism, and various microbial infections [11, 12, 14, 15]. Several studies have further documented its broad therapeutic applications in traditional healthcare systems [16, 17, 18].

In Nigeria, the plant is particularly valued in traditional pediatric medicine, where it is commonly used for treating childhood illnesses. Across African traditional medicinal practices, *B. dalzielii* has also been reported to be used in the management of diarrhea, malaria, vomiting, infections, and arthritis [19, 20, 21]. Additionally, fresh bark is traditionally administered as an emetic and is used in alleviating symptoms such as dizziness and heart palpitations [22]. In northwestern Nigeria, the bark is used specifically in the traditional treatment of arthritis [23]. In our previous work, we performed a comprehensive phytochemical characterization and evaluated the antioxidant activity of aqueous extracts obtained from the leaves, stem bark, and root bark of *B. dalzielii* using UHPLC-DAD-MS analysis and *in vitro* antioxidant assays [24]. The findings revealed a rich composition of bioactive phytochemicals, including phenolic compounds and flavonoids, which are known to contribute to various pharmacological effects [24].

Despite these promising reports, existing studies on *B. dalzielii* have primarily focused on antioxidant activity, antimicrobial screening, and anti-inflammatory effects using animal or general biochemical models. To date, there is limited scientific evidence evaluating the dermatological relevance of *B. dalzielii* extracts using human skin cellular models. Furthermore, no studies have comprehensively investigated the combined effects of *B. dalzielii* on keratinocyte-mediated inflammatory responses, wound-healing processes, extracellular matrix-degrading enzyme inhibition, and antimicrobial activity against clinically relevant skin pathogens. In particular, there is a lack of studies examining the effects of aqueous extracts of *B. dalzielii* leaves and stem bark on TNF-α/IFN-γ-stimulated HaCaT keratinocytes, as well as their inhibitory potential against collagenase, elastase, and hyaluronidase enzymes involved in skin tissue degradation. In this study, aqueous extracts were intentionally selected to reflect the traditional (folkloric) preparation methods of *Boswellia dalzielii*, where the plant is commonly administered as water-based decoctions or infusions. Although hydroalcoholic solvents may extract a broader range of phytochemicals, water use ensures that the evaluated biological activities remain directly relevant to its ethnopharmacological application.

Therefore, the present study aimed to scientifically validate the traditional use of *Boswellia dalzielii* in the management of skin infections and inflammatory skin disorders. Specifically, we evaluated the cytotoxicity and wound-healing potential of aqueous leaf and stem bark extracts using HaCaT keratinocyte models through cell viability and scratch assays. The anti-inflammatory activity of the extracts was assessed by measuring nitric oxide production and the secretion of pro-inflammatory cytokines IL-6 and IL-8 in TNF-α and IFN-γ-stimulated keratinocytes. In addition, the inhibitory effects of the extracts on extracellular matrix-degrading enzymes, including collagenase, elastase, and hyaluronidase, were investigated. Finally, the antibacterial activity of the extracts was evaluated against two clinically relevant skin and wound pathogens, *Staphylococcus aureus* ATCC® BAA1720 and *Pseudomonas aeruginosa* ATCC® 27853.

2. Materials and methods

2.1. Chemicals and reagents

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide (Suc-AAA-pNA) (Cat. No. M4765), human leukocyte elastase (Cat. No. 324681), hyaluronidase acid sodium (Cat. No. 53747), hyaluronidase from bovine testes (Cat. No. H3506), N-[3-(2-furyl)-acryloyl]-Leu-Gly-

Pro-Ala (FALGPA) (Cat. No. F5135), collagenase from *Clostridium histolyticum* (Cat. No. C0773-3KU), epigallocatechin gallate (Cat. No. E4143), tannic acid (Cat. No. 1401-55-4), neocuproine (Cat. No. M1501) were obtained from Sigma-Aldrich (St. Louis, MO, USA). High-grade methanol was obtained from Avantor, located in Gliwice, Poland. Enzyme-linked immunosorbent assay (ELISA) kits (for interleukin-8 (IL-8) and interleukin-6 (IL-6)) were purchased from BD Biosciences (Franklin Lakes, NY, USA). Ciprofloxacin was obtained from Polpharma (Starogard Gdański, Poland). Dulbecco's Modified Eagle Medium (DMEM) high glucose w/stable glutamine w/sodium pyruvate, Dulbecco's Phosphate Buffered Saline (DPBS) w/o Ca^{2+} and Mg^{2+}, foetal bovine serum (FBS), and penicillin-streptomycin solution were obtained from Biowest (Nuaillé, France). Recombinant human tumour necrosis factor-alpha (TNF-α) and recombinant human interferon-gamma (IFN-γ) were purchased from InvivoGen (Toulouse, France). Kinetex XB-C_{18} column was gotten from Agilent (Santa Clara, CA, USA). The Merck Millipore Simplicity UV system was employed to produce ultra-pure water.

2.2. Sample Collection

Professor Halima Mohammed Abba collected *Boswellia dalzielli* (*B. dalzielli*) Hutch leaves and stem bark (Fig. 1) in December 2023. The location of the collection was Gadam, Kwami Local Government Area, located in Gombe State in the northeastern region of Nigeria (11° 6' 6" E ·10.47484,11.10177·Aw: Tropical Savanna). In January 2024, Mr. Omotayo Felix Olorunfemi, who works in the herbarium section of the Department of Plant Science and Biotechnology at Ekiti State University, Ado Ekiti, was the one who authenticated the plant samples. Additionally, Mr. Omotayo Felix Olorunfemi deposited voucher specimens (UHAE 2024001a and UHAE 2024001b for leaves and stem bark, respectively) for future reference.

Fig. 1. Pictorial representation of leaf (BDL) and stem bark (BDSB) of *Boswellia dalzeilii*. (Adapted from: [25])

2.3. Sample preparation

Fresh leaves and stem bark of *B. dalzielii* were cleaned and air dried until constant weights were obtained. The leaves were homogenized using a blender (Vitamix 5200, 2-peak horsepower motor) while the stem bark was reduced to coarse powder using pestle and mortar, then pulverized using electric grinder. The plant samples were kept separately in an airtight container prior to the extraction.

2.4. Plant extracts preparation and extracts percentage yield

Separately, the ground plant samples (50 g each) were soaked in distilled water (1000 ml) for 24 hrs on a rotatory shaker set at 150 rpm. The products were filtered and then freeze-dried. It gave a yield of 15% and 15% (w/w) for leaf and stem bark, respectively. For the biological assay's parameters, 10 mg of the freeze-dried extracts were reconstituted in 1 mL of distilled water.

2.5. Studies on skin cell lines

2.5.1. Cell cultures

The human keratinocytes (HaCaT) cell line (Lonza Group Ltd, Basel, Switzerland) was cultured in a humidified incubator at 37°C with 5% CO_2. Cells were cultured in DMEM High Glucose Medium (w/L-Glutamine, w/Sodium Pyruvate) containing 10% fetal bovine serum and 1% penicillin and streptomycin solution.

2.5.2. Evaluation of the viability of HaCaT cell line

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was conducted to examine the effect of the extracts on the viability of the HaCaT cell line. The HaCaT cell line (2 x 10^4 per well) were cultured in a 48-well plate for 48-72 hrs until reaching 50-80% confluence. Appropriate concentrations of extracts (raw extracts: 25.00, 50.00, 100.00, and 200.00 µg/mL) which were dissolved in a medium, were added and left to incubate for 24 hrs. Vehicle (0.1% DMSO) served as the negative control, and 0.1% triton X-100 in DPBS (TX-100) was used as the positive control. The medium was subsequently aspirated, and DPBS was used to rinse the cells after which MTT powder dissolved in a medium (500 µg/mL) was added. After the incubation, the medium above the cells was aspirated, and water-insoluble dark blue formazan crystals were then dissolved in 300 µL of DMSO and agitated for 5 min. Absorbance was measured at 570 nm with a correction at 630 nm. Results were presented as a percentage of the control (medium without tested samples).

2.5.3. *In vitro* wound healing assay

The HaCaT cells (1.70 × 10^5 × mL^{-1}) were cultured in a 12-well plate for about 18 hrs until a confluent monolayer was formed. Afterwards, cells were rinsed with DPBS and incubated for additional 4 hrs with FBS-free medium. The cell single layer was scratched off with a yellow pipette tip, and the cells were washed with DPBS after aspirating the medium. Furthermore, the FBS-free medium, which contains the extracts (100 µg × mL^{-1}) was added to the cell. Photographs were taken at different hours (0 hrs, 24 hrs, and 48 hrs) after the application of the extract using a Nikon camera (Nikon Eclipse TS100, Nikon Instruments Inc., Melville, NY, USA, supported by NIS-Elements BR 3.22 Software). The results were presented as a wound closure ratio and the scratch

surface area at time of 0 hrs and after 24 hrs and 48 hrs of incubation with the tested extract were compared, with the control being the FBS-free medium.

$$\text{Wound closure (\%)} = \left(\frac{At0h - At}{At0h}\right) x\ 100$$

$$\text{Cell migration rate } \left(\frac{\mu m}{h}\right) = \left(\frac{Wi - Wf}{t}\right)$$

where 'At = 0' is the area of the initial wound just after scratching, 'At' is the wound area after "n" hrs of initial scratching, 'Wi' is the average of the initial wound width in µm, 'Wf' is the average of the final wound width in µm, and 't' is the time of the assay (in hrs) until the control wound closed.

2.5.4. TNF-α and IFN-γ stimulation

HaCaT cells ($2 \times 10^4 \times mL^{-1}$) were cultured to a confluence of 80% in a 24-well plate, after which the medium was aspirated and the cells rinsed with DPBS, then a fresh medium containing the sample at the appropriate extract concentrations (25, 50, and 100 µg/mL) was applied. To mimic an inflammatory microenvironment and induce the expression of pro-inflammatory mediators, the cells were stimulated with a mixture of TNF-α and IFN-γ (10 ng × mL^{-1} each) prepared in DPBS. Supernatants were aspirated after 24 h of incubation and kept at -20 ºC until needed. The cells treated with DPBS alone (without TNF-α and IFN-γ) served as the non-stimulated control.

2.5.5. Evaluation of IL-6 and IL-8 secretion

The level of cytokines (evaluation of IL-6 and IL-8 secretion) in the harvested supernatants was measured by using an Enzyme-Linked Immunosorbent Assay (ELISA) test kit according to the manufacturer's instructions (BD Biosciences, San Diego, CA, USA; Catalog Number: 555220 and 555244 for IL-6 and IL-8, respectively).

2.5.6. Evaluation of nitric oxide (NO) production

The harvested supernatants were used for nitrite estimation using the Griess reaction. Nitric oxide level was accurately measured following reduction of nitrate to nitrite using an improved Griess reagent according to the manufacturer's instructions (Sigma-Aldrich (St. Louis, MO, USA); Catalog Number: MAK454). Briefly, HaCaT cells ($2 \times 10^4 \times mL^{-1}$) were cultured to a confluence of 80% in a 24-well plate, after which the medium was aspirated and the cells rinsed with DPBS then pretreated with a fresh medium which contains the sample at the appropriate extract concentrations (25, 50, and 100 µg/mL) for six hrs and then incubated in the presence or absence of mixture of TNF-α and IFN-γ (10 ng × mL^{-1} each) prepared in DPBS for 24 h. The supernatant of the culture medium in each well was incubated with Griess reagent (20 mg/mL) for 15 min. The cells treated with DPBS alone (without TNF-α and IFN-γ) served as the control. The absorbance was measured at 540 nm within 30 min using a Synergy 4 BioTek microplate reader (Vinooski, VT, USA).

2.6. Elastase inhibitory assay

Elastase inhibitory activity was assessed using N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide (Suc-AAA-pNA) (1 mM) as substrate [26]. The reaction mixture initially contained HEPES buffer (0.1M, pH 7.5), followed by the addition of the test extract or reference standard inhibitors (ursolic acid and quercetin) at varying concentrations (0 – 100 µg/mL). Elastase enzyme (1 µg/mL) was subsequently added, and the mixture was pre-incubated for 20 mins at room temperature to allow enzyme–inhibitor interaction. The reaction was initiated by the addition of the substrate (Suc-AAA-pNA) and further incubated for 40 mins at 37 °C. Elastase activity was determined by measuring the release of p-nitroaniline at 405 nm using a spectrophotometer. Percentage inhibition of elastase activity was calculated relative to the control reaction without inhibitor, and IC_{50} values were determined from dose–response curves generated using the extract concentrations.

2.7. Hyaluronidase inhibitory activity assay

The inhibitory activity of Hyaluronidase was carried out following the method of Jakupović et al. [27] with a little modification. The test solution or positive control (tannic acid) with a varying concentration range (0 – 70.42 µg/mL) and hyaluronidase solution (20 µL, 4 mg/mL) were mixed and incubated at 37 °C. $CaCl_2$ solution (40 µL, 12.5 mM) was added after 20 mins and was further incubated for 20 mins after which 50 µL of sodium hyaluronate solution (3.5 mg/mL) was added and incubated for 40 mins at 37 ºC while constantly shaking it. The reaction was stopped by the addition of NaOH and sodium tetraborate, and then heated for 3 mins at 100 °C. Subsequently, 160 µL of p-dimethylaminobenzaldehide reagent (DMABA) (0.4 g DMABA dissolved in 35 mL of 100% acetic acid and 5 mL of 10 M HCl) was added to the mixture and then incubated at 37 °C, after 20 min absorbance was measured at 585 nm.

2.8. Collagenase inhibitory activity assay

The UV–visible spectra of all test samples were acquired using a Synergy 4 BioTek microplate reader (Vinooski, VT, USA) to confirm the lack of spectral interference or changes in absorption maxima prior to conducting the enzymatic screening. The activity of collagenase was evaluated using a modified spectrophotometric method optimised for microplate analysis [28]. The reaction was performed in a 50 mM Tricine buffer (pH 7.5) containing 400 mM NaCl and 10 mM $CaCl_2$. Collagenase derived from ***Clostridium histolyticum*** (ChC; EC 3.4.23.3) was prepared in buffer to achieve an initial activity of 0.8 U/mL (2.0 U/mL as indicated in our proprietary reagent), in accordance with the manufacturer's specifications (Merck, Cat. No. C0773-3KU). The synthetic substrate N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA) (Merck, Cat. No. F5135) was solubilised in Tricine buffer to achieve a final concentration of 1.0 mM. Test samples were pre-incubated with the enzyme for 60 mins before substrate addition to commence the reaction. Each well (150 µL final volume) had 1.0 mM FALGPA, 0.1 U ChC, and test sample concentrations ranging from 0 to 50 µg. Distilled water functioned as the negative control. Absorbance at 335 nm was recorded immediately upon substrate addition and continuously for 20 mins using a Synergy 4 BioTek microplate reader (Vinooski, VT, USA) with a Nunc 96-well plate. Epigallocatechin gallate (EGCG; Merck, Cat. No. E4143) was utilised as the positive reference at concentrations of 250 µM.

2.9. Antibacterial activity assay

The antibacterial potential of the extracts was assessed using *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC BAA1720 as model skin-

associated bacterial strains. For inoculum preparation, each bacterial strain was first cultured overnight on Brain Heart Infusion (BHI) agar at 37 °C. A bacterial suspension equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) was then prepared and further diluted at a ratio of 1:99 in BHI broth for subsequent experiments. Stock solutions of the extracts were prepared in sterile distilled water and sterilized using 0.22 µm PVDF syringe filters prior to testing. Defined volumes of the prepared bacterial suspensions were transferred into sterile 96-well microplates containing serial dilutions of the extracts (0.02–10 mg/mL), achieving a final bacterial density of 5×10^5 CFU/mL in each well. The microplates were sealed with adhesive films and incubated at 37 °C in a microplate reader for 20 hrs. Bacterial growth was monitored by measuring optical density at 600 nm (OD_{600}) at 30-min intervals throughout the incubation period. All experiments were conducted in three independent biological replicates.

2.10. Statistical analysis

All measurements were conducted using three independent experiments, each performed in triplicate. Data are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using Prism version 8.0.2 software developed by GraphPad Software, Inc (San Diego, California). Differences between groups were evaluated using two-way ANOVA followed by Tukey's multiple comparison test. Statistical significance was established at $p < 0.05$. The SC_{50} and IC_{50} values were determined from concentration–response inhibition curves generated by fitting the experimental data using GraphPad Prism version 8.0.2 for Windows.

3. Results

3.1. Effect of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii* on viability of HaCaT cell line

Exposure of HaCaT keratinocytes to aqueous extracts of *Boswellia dalzielii* leaves (BDL) and stem bark (BDSB) did not induce noticeable morphological alterations compared to untreated control cells (Fig. 2A). The cells retained their normal polygonal morphology and confluency across all tested concentrations (25–200 µg/mL). In contrast, cells treated with the positive control, 0.1% Triton X-100 (TX-100), showed evident morphological deterioration, characterized by loss of cellular integrity and reduced cell density. Quantitative assessment using the MTT assay demonstrated that both BDL and BDSB extracts maintained high cell viability in HaCaT cells (Fig. 2B). The untreated control exhibited a viability of 100.20 ± 0.75%, whereas TX-100 significantly reduced cell viability to 6.10 ± 0.61% ($p < 0.05$). Treatment with BDL extract resulted in cell viability ranging from 99.14 ± 1.40% to 100.67 ± 0.55% across concentrations of 25, 50, 100, and 200 µg/mL. Similarly, BDSB extract maintained viability between 98.75 ± 1.02% and 99.53 ± 0.46% within the same concentration range. No statistically significant ($p < 0.05$) reduction in cell viability was observed for either extract when compared to the untreated control, whereas both extracts significantly ($p < 0.05$) differed from the TX-100-treated group. These findings indicate that aqueous leaf and stem bark extracts of *Boswellia dalzielii* are non-cytotoxic to HaCaT keratinocytes at the tested concentrations.

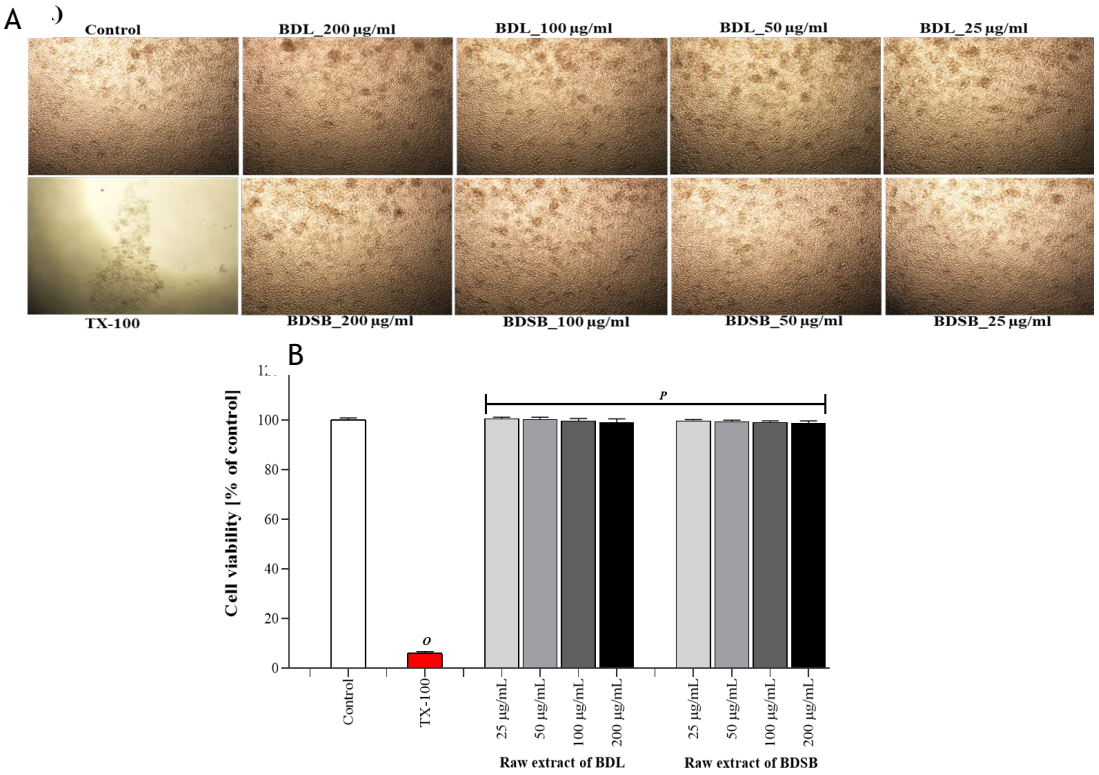

Fig. 2. (A) Morphology of HaCaT cell line following MTT viability assay at different concentrations of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii*. (B) Cell viability (%) determined by MTT assay after exposure of HaCaT cell line to varying concentrations of aqueous leaf and stem bark extracts of *Boswellia dalzeilii*. Data are presented as mean ± standard deviation (SD) of three independent experiments. Statistical significance was indicated as follows: $^{o}p < 0.05$ compared to the control, and $^{P}p < 0.05$ compared to TX-100. TX-100 served as the positive control (0.1% Triton X-100).

3.2. Wound healing assay

The effect of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii* on HaCaT keratinocyte migration was assessed using an *in vitro* scratch wound assay (Fig. 3). Representative micrographs (Fig. 3A) showed progressive wound closure over time in all groups, with visibly enhanced cell migration in extract-treated cells compared to the control at both 24 and 48 hrs post-scratch. Quantitative analysis revealed that both extracts significantly enhanced cell migration compared to the control. Quantitative analysis of wound closure (Fig. 3B) demonstrated that at 24 hrs, the control group exhibited a wound closure of 32.80 ± 2.24%, whereas BDSB treatment resulted in significantly ($p < 0.05$) higher wound closure (81.76 ± 0.56%) compared to the control and BDL. BDL-treated cells showed moderate wound closure (66.17 ± 1.02%), which was significantly ($p < 0.05$) greater than the control but significantly ($p < 0.05$) lower than BDSB. At 48 hrs, wound closure increased in all groups, with the control reaching 64.49 ± 1.18%. BDSB-treated cells achieved complete wound closure (100 ± 0.00%), which was significantly ($p < 0.05$) higher than both the control and BDL. BDL treatment resulted in a wound closure of 84.07 ± 0.48%, remaining significantly ($p < 0.05$) higher than the control but significantly ($p < 0.05$) lower than BDSB. The average cell migration rate (Fig. 3C) followed a similar trend. BDSB-treated cells exhibited the highest migration rate (8.24 ± 0.25 µm/h), which was significantly ($p < 0.05$) greater than both the control and BDL. BDL treatment increased the migration rate to 6.93 ± 0.24 µm/h compared to the control (5.31 ± 0.27 µm/h) but remained significantly ($p < 0.05$) lower than BDSB. The results indicate that both aqueous leaf extracts enhanced keratinocyte migration and wound closure relative to the control, with BDSB demonstrating a significantly ($p < 0.05$) stronger effect than BDL across all evaluated parameters.

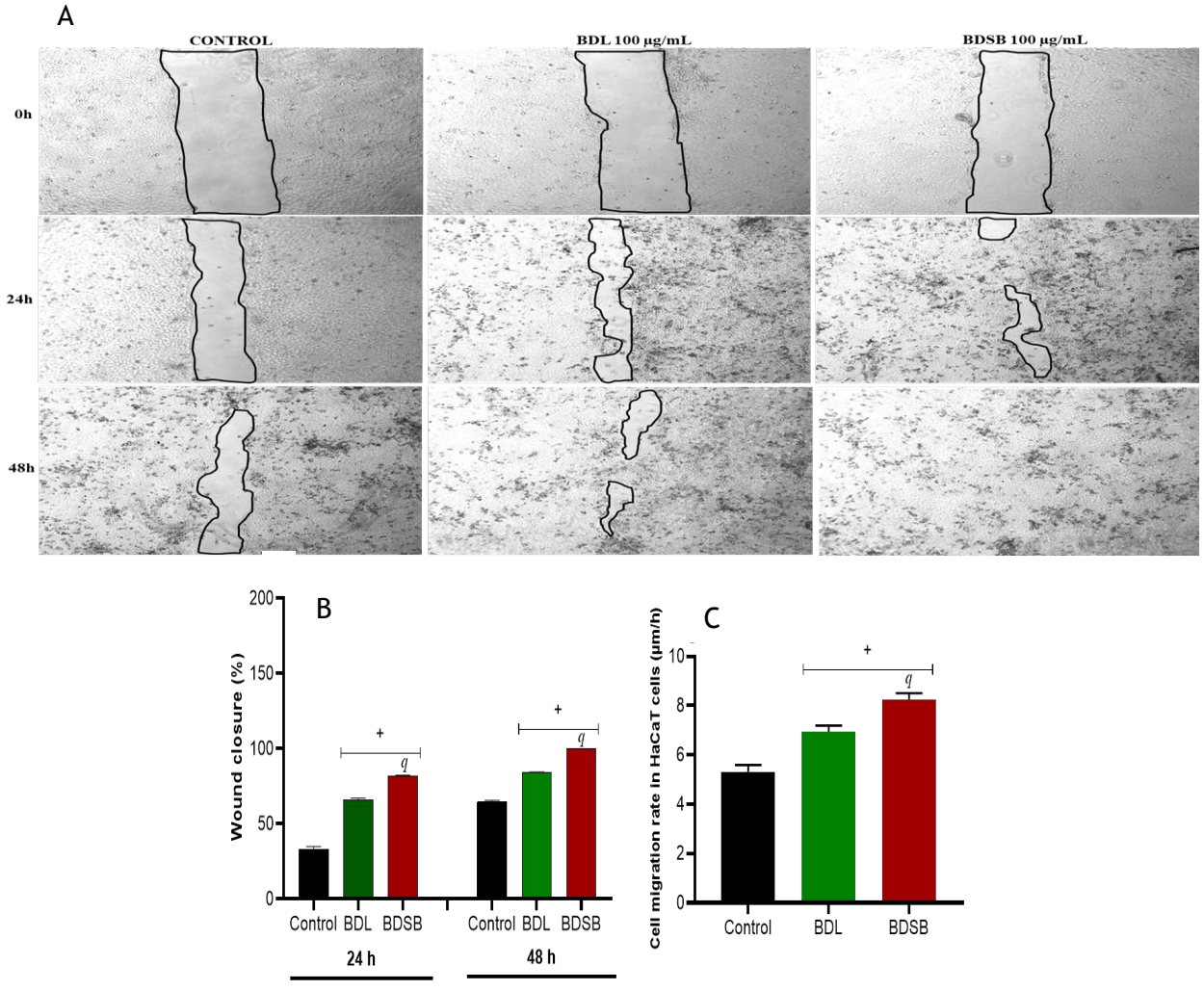

Fig. 3. The effect of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii* on migrating HaCaT keratinocytes to the scratch site. (A) representative photographs for each tested group at 0, 24 and 48 h after scratching. (B) Percentage of wound closure for treatment and the control in HaCaT cells at: 24 and 48 h. (C) Bar graph of average cell migration rate (µm/h) after treatment and the control in HaCaT cells. The results are presented as the mean ± SD of three experiments. Statistical difference was accepted as follows: $^{+}p < 0.05$ indicates statistically significant different from control; $^{q}p < 0.05$ indicates statistically significant different from BDL.

3.3. Effects of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii* on IL-6 and IL-8 secretion by TNF-α/IFN-γ-stimulated HaCaT keratinocytes

As shown in Fig. 4A, stimulation of HaCaT keratinocytes with TNF-α and IFN-γ (ST) significantly ($p < 0.05$) increased IL-6 secretion to 126.59 ± 0.41 pg/mL of stimulated control compared with non-stimulated cells (NST, 16.09 ± 0.62 pg/mL). Treatment with BDL resulted in a concentration-dependent reduction in IL-6 levels to 109.00 ± 0.77, 73.19 ± 0.67 pg/mL, and 53.68 ± 0.68 pg/mL at 25, 50, and 100 µg/mL, respectively, and was significantly ($p < 0.05$) different from ST. Similarly, BDSB decreased IL-6 secretion to 81.80 ± 0.72, 66.28 ± 0.71, and 46.79 ± 0.83 pg/mL at 25, 50, and 100 µg/mL, respectively, showing a stronger inhibitory effect than FEL at equivalent concentrations. Urolithin A, used as a positive control, further suppressed IL-6 levels to 63.31 ± 0.50, 45.36 ± 0.31, and 33.36 ± 0.68 pg/mL at 12.5, 25, and 50 µM, respectively ($p < 0.05$ vs. ST). For IL-8 secretion (Fig. 4B), ST induced a maximal increase to 81.96 ± 0.17 pg/mL relative to NST (10.90 ± 0.15 pg/mL, $p < 0.05$). BDL reduced IL-8 levels in a dose-dependent manner to 66.34 ± 0.17, 47.55 ± 0.11, and 27.46 ± 0.15 pg/mL at 25, 50, and 100 µg/mL, respectively ($p < 0.05$ vs. ST). BDSB was more potent, decreasing IL-8 secretion to 55.07 ± 0.60, 33.62 ± 0.20, and 19.41 ± 0.21 pg/mL at corresponding concentrations. Urolithin A exhibited the strongest suppression of IL-8, reducing secretion to 39.82 ± 0.36, 26.78 ± 0.22, and 15.46 ± 0.15 pg/mL at 12.5, 25, and 50 µM, respectively ($p < 0.05$ vs. ST). Both BDL and BDSB significantly attenuated TNF-α/IFN-γ-induced pro-inflammatory cytokine secretion in HaCaT keratinocytes in a concentration-dependent manner, with BDSB showing slightly stronger inhibition than BDL at equivalent doses.

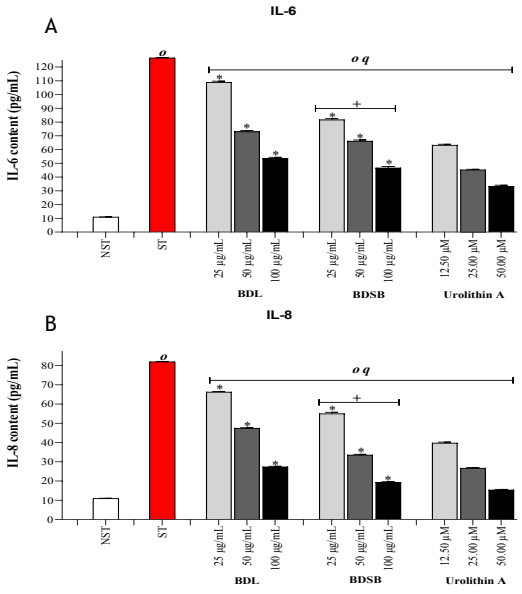

Fig. 4. Effect of 24 hrs incubation with aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii* on the secretion of IL-6 content (A) and IL-8 content (B) by HaCaT keratinocytes stimulated with TNF-α and IFN-γ. The results are presented as the mean ± SD of three experiments. [o]Statistically ($p < 0.05$) significant relative to NST; [q]Statistically ($p < 0.05$) significant relative to ST; [*]Statistically ($p < 0.05$) significant relative to Urolithin A; [+]Statistically ($p < 0.05$) significant relative to BDL.

3.4. Effects of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii* on *TNF-α/IFN-γ*-induced nitric oxide (NO) production in HaCaT keratinocytes

Stimulation of HaCaT cells with TNF-α/IFN-γ significantly increased nitric oxide (NO) production compared with untreated control cells. The NO level in TNF-α/IFN-γ-induced cells (7.64 ± 0.17 µM) was significantly higher ($p < 0.05$) than that of the control group (3.08 ± 0.08 µM) (Fig. 5), confirming successful induction of an inflammatory response. Treatment with aqueous leaf extract of *Boswellia dalzielii* (BDL) resulted in a concentration-dependent reduction in NO production. BDL treatment at 25, 50 and 100 µg/mL reduced NO levels to 7.39 ± 0.28, 6.62 ± 0.26 and 5.53 ± 0.21 µM, respectively. However, the reductions observed at 50 and 100 µg/mL were significantly lower ($p < 0.05$) than the TNF-α/IFN-γ-induced untreated group, whereas the reduction at 25 µg/mL showed minimal inhibitory effect (Fig. 5). Similarly, aqueous stem bark extract of *Boswellia dalzielii* (BDSB) markedly suppressed NO production in a concentration-dependent manner. BDSB treatment at 25, 50 and 100 µg/mL significantly decreased NO levels to 6.55 ± 0.14, 4.80 ± 0.13 and 3.95 ± 0.17 µM, respectively, relative to the TNF-α/IFN-γ-induced untreated group ($p < 0.05$). Furthermore, BDSB produced a significantly ($p < 0.05$) greater decrease in NO levels compared with BDL at corresponding concentrations (Fig. 5). Both leaf and stem bark extracts of *Boswellia dalzielii* significantly attenuated TNF-α/IFN-γ-induced NO production in HaCaT cells, with the stem bark extract exhibiting stronger anti-inflammatory activity than the leaf extract.

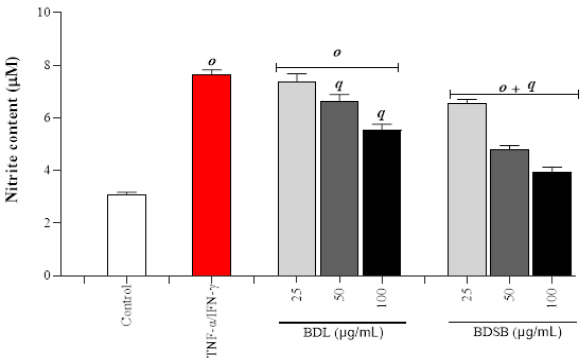

Fig. 5. Effect of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii*.on TNF-α/IFN-γ-induced nitric oxide (NO) content in HaCaT cells. The results are presented as the mean ± SD of three experiments. [o]Statistically ($p < 0.05$) significant relative to untreated control cells; [q]Statistically ($p < 0.05$) significant relative to TNF-α/IFN-γ alone. [+]Statistically ($p < 0.05$) significant relative to BDL.

3.5. Collagenase inhibitory activity

The time-course analysis at 50 µg/mL (Fig. 6A) shows that both BDL and BDSB extracts progressively inhibited collagenase activity over 20 mins, with BDSB demonstrating slightly higher inhibition than BDL at most time points. The positive control, EGCG, exhibited the strongest inhibition throughout the measurement period. Concentration-dependent assessment (Fig. 6B) revealed

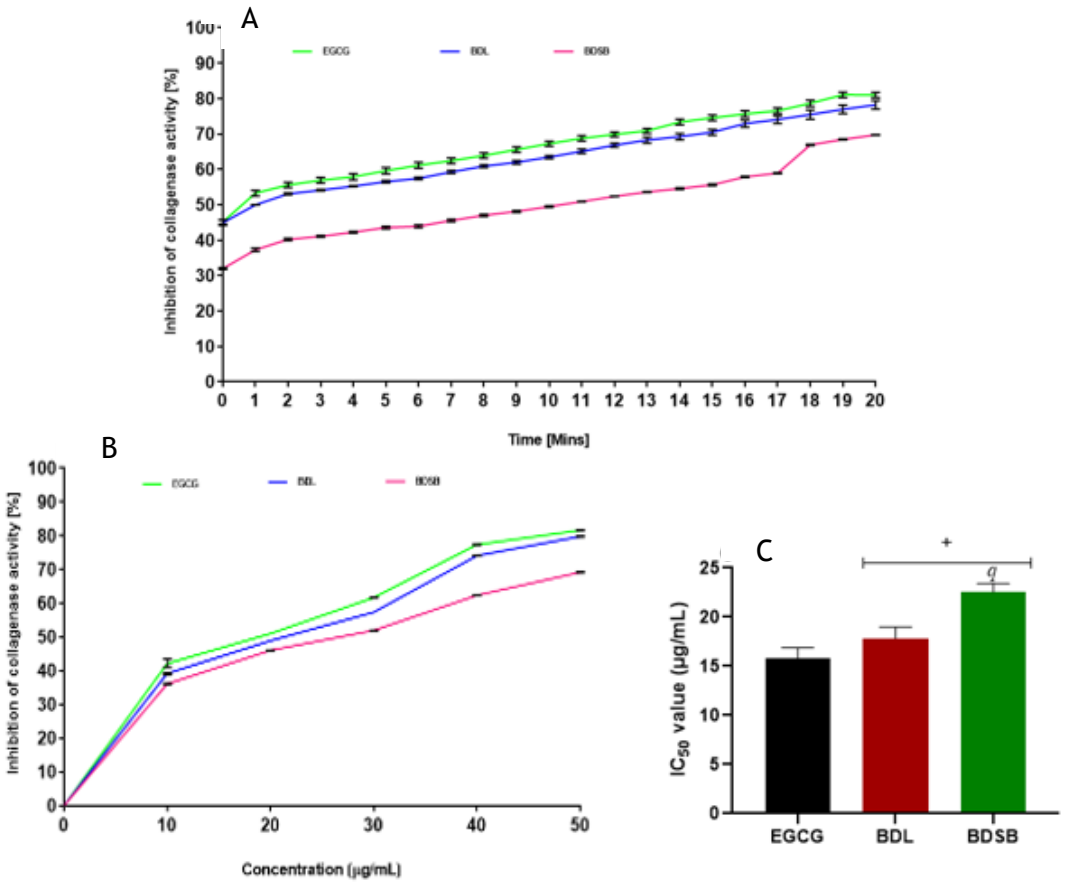

Fig. 6. Collagenase inhibitory activity of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii*. (A) Percentage inhibition at 50 µg/mL measured over 20 mins at 1-minute intervals; (B) Percentage inhibition at varying concentrations (0–50 µg/mL) measured at 20 mins; (C) IC_{50} values for collagenase inhibition by the extracts. The results are presented as the mean ± SD of three experiments. Statistical difference was accepted as follows: $^{+}p < 0.05$ indicates statistically significant different from control; $^{q}p < 0.05$ indicates statistically significant different from BDL.

that both BDL and BDSB inhibited collagenase activity in a dose-dependent manner, with inhibition increasing from 0 to 50 µg/mL. At 50 µg/mL, EGCG achieved a higher percentage of inhibition (81.02%) compared to BDSB (69.73%) and BDL (78.24%). The IC_{50} values (Fig. 6C) further support these observations. EGCG exhibited the strongest collagenase inhibition with an IC_{50} of 15.82 ± 1.06 µg/mL, followed by BDL (17.84 ± 1.11 µg/mL) and BDSB (22.55 ± 0.85 µg/mL). Statistical analysis indicated that both extracts significantly inhibited collagenase activity compared to the control ($p < 0.05$), and BDSB showed a significant difference compared to BDL ($p < 0.05$). These results indicate that aqueous extracts of *B. dalzeilii*, particularly from stem bark, possess collagenase inhibitory activity, suggesting potential utility in anti-aging and tissue-protective applications where collagen degradation is a concern.

3.6. Hyaluronidase inhibitory activity

The hyaluronidase inhibitory activities of BDL and DBSB were assessed at varying concentrations. As shown in Fig. 7A, the percentage inhibition of hyaluronidase increased with extract concentration (0 – 100 µg/mL), with BDSB showing significantly higher inhibition than BDL at all concentrations used. Fig. 7B depicts the IC_{50} values for hyaluronidase inhibition. BDL showed an IC_{50} of 38.55 ± 0.99 µg/mL, BDSB had 25.56 ± 1.05 µg/mL, and the reference compound tannic acid had 21.33 ± 1.07 µg/mL. Statistical analysis indicated that both BDL and BDSB's inhibitory activities were significantly ($p < 0.05$) lower than tannic acid, whereas BDSB exhibited significantly ($p < 0.05$) higher potency than BDL. These results suggest that both *Boswellia dalzeilii* extracts can inhibit hyaluronidase, with BDSB demonstrating the most potent activity.

3.7. Elastase inhibitory activity

The elastase inhibitory activities of BDL and BDSB were assessed at varying concentrations. As shown in Fig. 8A, the percentage inhibition of elastase increased with extract concentration (0–100 µg/mL), with BDSB showing significantly higher inhibition than BDL at all tested concentrations. At the highest concentration evaluated, BDSB achieved a higher percentage inhibition (71.50%) compared to BDL (65.55%), approaching that of the reference compound quercetin (75.63%), but remaining lower than ursolic acid (87.18%). The IC_{50} values (Fig. 8B) further support these observations. Ursolic acid demonstrated the strongest elastase inhibition, with an IC_{50} value of 19.95 ± 0.47 µg/mL, indicating the highest potency among the tested samples, followed by quercetin (27.91 ± 0.84 µg/mL). BDSB also exhibited notable inhibitory activity, with an IC_{50} value of 41.07 ± 1.00 µg/mL, whereas BDL showed the lowest potency (65.55 ± 1.04 µg/mL). These results suggest that the stem bark extract of *Boswellia dalzielii* possesses stronger elastase inhibitory activity than the leaf extract, although both extracts remain less potent than the reference compounds.

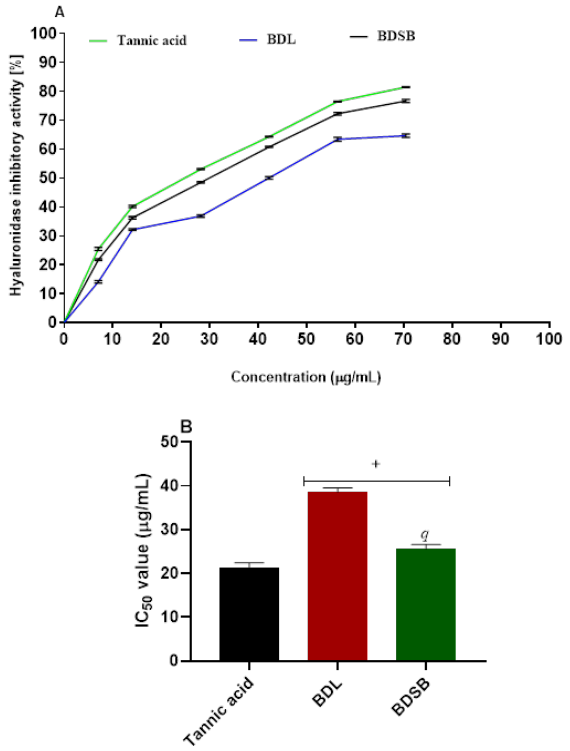

Fig. 7. (A). Inhibitory effect of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii*.on hyaluronidase activity. (B) IC_{50} values of inhibition of hyaluronidase activity by the extracts. The results are presented as the mean ± SD of three experiments. Statistical difference was accepted as follows: $^{+}p < 0.05$ indicates statistically significant different from control; $^{q}p < 0.05$ indicates statistically significant different from BDL.

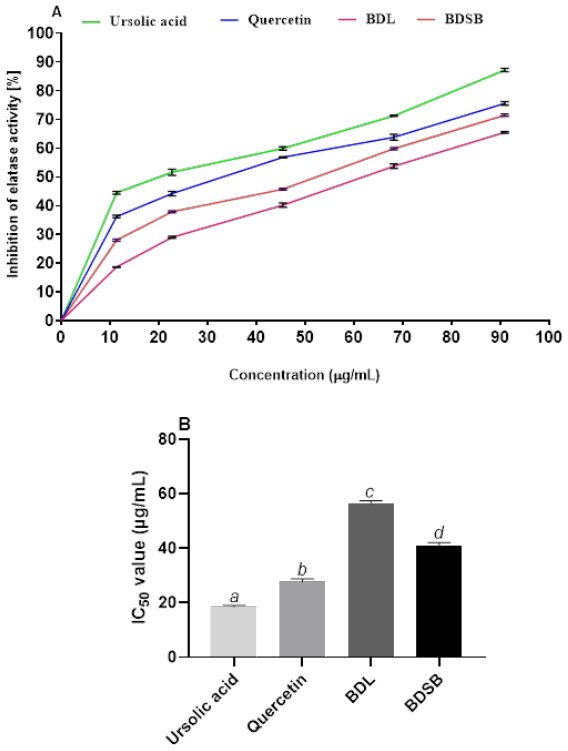

Fig. 8. (A). Inhibitory effect of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii* on elatase activity. (B) IC_{50} values of inhibition of elatase activity by the aqueous leaf extracts. Values with the same superscript letters are not significantly ($P < 0.05$) different. Data are presented as mean ± standard deviation (SD) of three independent experiments.

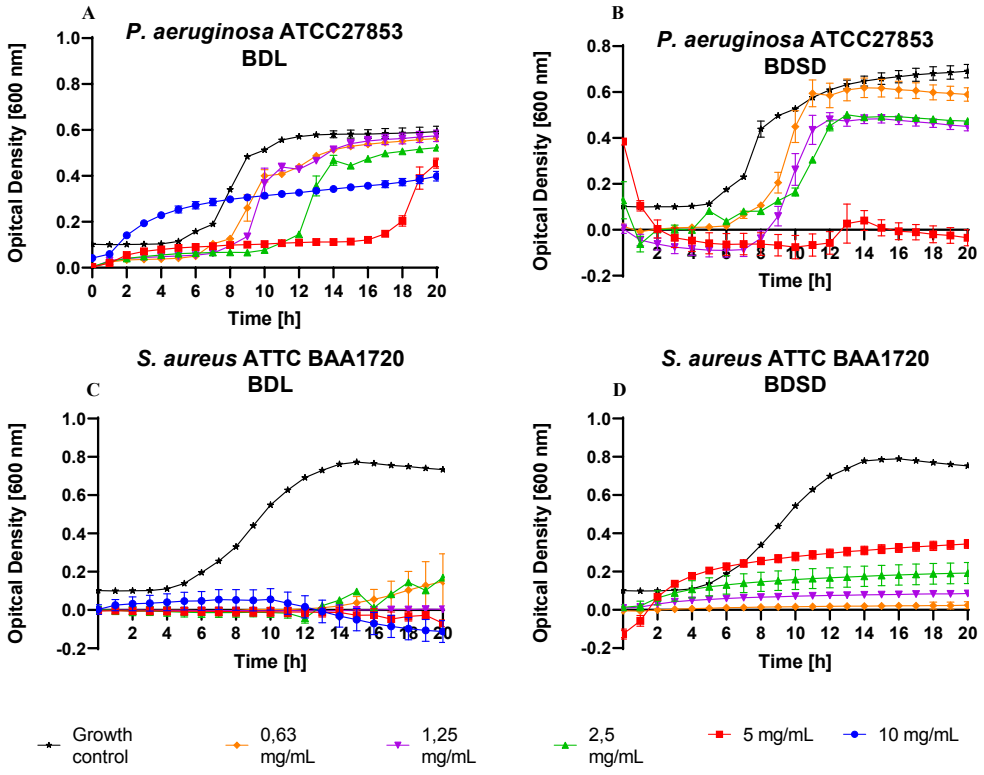

Fig. 9. Growth curves of tested bacteria treated with aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii*.

3.8. Antibacterial effects of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzeilii*

In *P. aeruginosa* ATCC 27853, both BDL and BDSD exhibited limited antibacterial activity and affected bacterial growth only at the highest tested concentration (MIC = 10 mg/mL). At this concentration, growth was strongly suppressed throughout the incubation period, whereas lower concentrations produced minimal or no changes in growth kinetics or maximal OD_{600} compared to the untreated control (Fig. 9). In contrast, *S. aureus* ATCC BAA-1720 was highly susceptible to both extracts. Growth inhibition was observed at low concentrations, with an MIC of 0.31 mg/mL for both BDL and BDSD, resulting in complete suppression of bacterial growth over time. Measured OD_{600} values under inhibitory conditions did not approach zero due to precipitation of the extracts, rather than residual bacterial growth, as indicated by stable OD_{600} readings over time and the absence of a growth curve shape characteristic of bacterial proliferation (Fig. 9).

4. Discussion

Assessment of cytotoxicity is an essential prerequisite in evaluating the therapeutic potential of plant extracts intended for dermatological application [29]. In the present study, aqueous extracts of *B. dalzielii* leaves and stem bark maintained high viability of HaCaT keratinocytes across all tested concentrations (25–200 µg/mL), with cell viability values comparable to untreated control cells. Microscopic examination further confirmed the preservation of normal keratinocyte morphology and confluency following extract exposure, whereas the positive control (Triton X-100) caused severe cellular damage.

These findings suggest that both extracts exhibit excellent cytocompatibility with human keratinocytes, supporting their suitability for topical therapeutic development. The absence of cytotoxicity observed in this study suggests that the extracts do not contain harmful constituents at the tested concentrations. This finding is consistent with previous reports indicating that *B. dalzielii* contains phenolic compounds and flavonoids, which are generally associated with low toxicity and potential protective effects against oxidative stress and cellular damage [19, 24, 30, 31].

Previous studies investigating the pharmacological properties of *Boswellia* genus have demonstrated similar cytoprotective properties in cell-based systems. For instance, extracts from *Boswellia serrata* have demonstrated that cell viability of human peripheral blood mononuclear cells and RAW 264.7 macrophages were not affected at concentrations up to 5 µg/ml [32]. Despite these reports, studies evaluating cytocompatibility of *B. dalzielii* using human skin-derived cell lines remain scarce. To the best of current knowledge, this study provides the first report demonstrating the safety of aqueous leaf and stem bark extracts of *B. dalzielii* in HaCaT keratinocytes, thereby providing new evidence supporting its traditional dermatological applications.

The observed cytocompatibility is consistent with ethnomedicinal reports describing the topical application of *B. dalzielii* preparations for treating wounds, septic sores, and inflammatory skin conditions in several African countries [10, 11, 12, 13]. Traditional remedies intended for wound healing require preservation of keratinocyte viability and function, as keratinocytes play crucial roles in re-epithelialization and barrier restoration during tissue repair [33]. The ability of the extracts to maintain keratinocyte integrity suggests that the plant may support cutaneous regeneration processes.

Maintenance of keratinocyte viability is essential for effective wound healing, as these cells regulate inflammatory signaling and contribute to tissue regeneration. The preserved cell morphology observed in this study further indicates that the extracts do not interfere with cellular adhesion or structural organization, both of which are critical for epithelial repair. These findings support the potential application of *B. dalzielii* extracts in topical formulations for wound management. Wound healing is a coordinated multistage biological process involving inflammation, tissue formation (including keratinocyte migration and proliferation), and remodeling, culminating in the restoration of skin integrity and function. Keratinocytes, the predominant epidermal cells, play a crucial role in re-epithelialization by migrating to cover the wound bed and re-establish the epidermal barrier.

Boswellia species (family Burseraceae) are well known in traditional medicine for their anti-inflammatory, antimicrobial, and tissue repair properties. Several species, including *Boswellia frereana*, *B. serrata*, and *B. carterii*, have demonstrated wound-healing effects *in vitro* and *in vivo*, such as enhanced cell migration, fibroblast proliferation, anti-inflammatory activity, and accelerated wound closure [34, 35]. However, the specific effects of *Boswellia dalzielii* extracts on human keratinocyte migration and wound closure remain insufficiently explored, highlighting the novelty of the present study.

In this study, both aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzielii* enhanced HaCaT keratinocyte migration compared to control cells in an *in vitro* scratch wound model. Microscopic observations revealed accelerated wound gap reduction over 24 and 48 h in extract-treated groups, with BDSB consistently exhibiting the greatest effect, achieving complete closure by 48 h, while BDL induced moderate but significantly greater wound closure than the control. Quantitative analysis further confirmed this trend, with BDSB demonstrating the highest wound closure percentages and migration rates at all time points, followed by BDL and control. These findings indicate a strong pro-migratory effect of the extracts, particularly the stem bark extract.

These observations are consistent with previous studies on other *Boswellia* species, where extracts have been shown to promote cellular activities associated with wound repair. For example, extracts of *B. frereana* have been reported to enhance HaCaT migration and fibroblast proliferation, along with anti-inflammatory effects that support wound healing [34]. Similarly, *B. serrata* extracts have been shown to stimulate dermal fibroblast migration and proliferation in scratch wound models [36]. Although these studies primarily examined different *Boswellia* species, the general trend of enhanced cell migration and wound closure from *Boswellia* extracts is consistent with the current observations for *B. dalzeilii*. These data indicate that *B. dalzeilii* aqueous extracts positively

influence keratinocyte migration and wound closure *in vitro*, consistent with wound-modulating effects reported for other *Boswellia* species. The enhanced migration observed for BDSB compared to BDL may be associated with differences in their phytochemical profiles. Our previous UHPLC-DAD-MS analysis of *B. dalzielii* aqueous extracts showed that both leaves and stem bark contain diverse secondary metabolites, including phenolic acids, flavonoids, and stilbenoid-related constituents. Notably, major compounds such as gallic acid, chlorogenic acid, ellagic acid, quercetin, astragalin, and methoxyresveratrol were identified, with variation in their distribution between plant parts [24]. These compounds have been widely associated with biological activities relevant to wound healing, including the modulation of cellular migration and tissue repair processes.

Inflammation is a central feature of many skin disorders and delayed wound healing, often driven by pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8). Keratinocytes, the predominant cells of the epidermis, not only form a physical barrier but also actively participate in inflammatory signaling when stimulated by cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [37]. Excessive IL-6 and IL-8 secretion by keratinocytes contributes to sustained inflammation and can impair tissue repair processes. Natural products with anti-inflammatory properties are of great interest for therapeutic modulation of cytokine responses in skin inflammation [37]. Extracts from *Boswellia* species have been investigated for anti-inflammatory effects in various systems. However, specific reports on *Boswellia dalzeilii*'s effects on keratinocyte cytokine secretion are limited, and available evidence on this species is primarily from systemic inflammation or animal models rather than skin cells.

Stimulation of HaCaT keratinocytes with TNF-α and IFN-γ (ST) significantly upregulated IL-6 and IL-8 secretion relative to non-stimulated controls, confirming activation of an inflammatory phenotype typical of cytokine-driven skin inflammation models. Treatment with *Boswellia dalzeilii* leaf (BDL) and stem bark (BDSB) extracts produced concentration-dependent reductions in both IL-6 and IL-8 secretion. At equivalent doses, BDSB exhibited stronger inhibition of pro-inflammatory cytokines than BDL, indicating differential bioactivity between the plant parts. This variation may be linked to differences in their phytochemical profiles, as demonstrated by our UHPLC-DAD-MS analysis, which showed that stem bark and leaves share overlapping but not identical sets of identified secondary metabolites [24]. The dose-dependent attenuation of IL-6 by BDL (from ~109 to ~53 pg/mL) and BDSB (from ~81.8 to ~46.8 pg/mL) suggests modulation of inflammatory signaling pathways triggered by TNF-α/IFN-γ. A similar pattern was seen with IL-8, where both extracts significantly reduced cytokine levels compared to stimulated controls, and BDSB achieved greater suppression across all concentrations tested. These inhibitory effects are consistent with the known anti-inflammatory properties attributed to *Boswellia* extracts, in which bioactive components such as boswellic acids have been shown to downregulate pro-inflammatory cytokines including IL-6 and TNF-α in various cellular and animal models [38].

Although there are not well-established prior studies specifically on *B. dalzeilii* effects in TNF-α/IFN-γ-stimulated keratinocytes, related research on *Boswellia* species provides comparative context. For example, Alqarni [35] reported that ethanolic extracts and essential oil of *Boswellia frereana*, as well as its constituents epilupeol and α-pinene, significantly reduced protein levels of several inflammatory mediators, including IL-1β, IL-6, IL-8, and TNF-α in LPS-stimulated HaCaT cells. This aligns with the present findings for *B. dalzeilii*, suggesting that *Boswellia* species contain phytochemicals capable of attenuating keratinocyte-mediated cytokine secretion [35].

In other studies, α-boswellic acid from *B. serrata* reduced TNF-α/IFN-γ-induced IL-6 and IL-8 expression in HaCaT cells, along with inhibition of MAP kinase and NF-κB pathways, which are critical mediators of cytokine-driven inflammatory responses [39]. This supports a possible mechanism whereby *B. dalzeilii* extracts may similarly influence key signaling pathways downstream of TNF-α/IFN-γ stimulation, leading to reduced cytokine production. In animal models, *B. dalzeilii* extracts have demonstrated systemic anti-inflammatory effects, such as decreasing IL-6 levels in diabetic rats, which is consistent with the notion that this species possesses active constituents capable of modulating inflammatory mediators [40].

The use of Urolithin A as a positive control further highlights the relevance of regulating IL-6 and IL-8 in inflammatory models, as this compound is known to suppress pro-inflammatory cytokine secretion. Both *B. dalzeilii* extracts achieved significant suppression of cytokine levels compared with stimulated controls, demonstrating that plant extracts can modulate inflammatory responses in keratinocytes at pharmacologically relevant concentrations. The stronger inhibitory activity seen with BDSB versus BDL may reflect differences in extract composition [24].

Inflammation represents a complex biological response to tissue injury and microbial invasion, involving coordinated signaling between cytokines, reactive oxygen species, and reactive nitrogen intermediates such as nitric oxide (NO) [41]. NO is synthesized primarily by inducible nitric oxide synthase (iNOS) in response to pro-inflammatory mediators including tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) [42]. Although physiologically important in host defense and cellular signaling, excessive NO production contributes to oxidative stress, inflammatory tissue damage, and the progression of dermatological disorders [43]. The TNF-α/IFN-γ-induced NO production model in HaCaT keratinocytes is therefore widely employed to simulate inflammatory responses associated with skin diseases and to evaluate potential anti-inflammatory agents.

In the present study, stimulation of HaCaT cells with TNF-α/IFN-γ significantly elevated NO production compared with untreated control cells, confirming successful induction of an inflammatory state. Treatment with aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzielii* significantly attenuated NO levels in a concentration-dependent manner, with the stem bark extract exhibiting greater NO-reducing capacity than the leaf extract at corresponding concentrations. These findings demonstrate that *B. dalzielii* possesses considerable anti-inflammatory potential and suggest

differential distribution of bioactive compounds between plant parts.

The anti-inflammatory and wound-healing (*in vitro* scratch assay) activities observed in the present study may be linked to modulation of key intracellular signaling pathways involved in skin inflammation and tissue repair. In particular, the reduction of pro-inflammatory mediators such as IL-6, IL-8, and nitric oxide suggests possible interference with the NF-κB signaling pathway, which regulates the transcription of inflammatory genes, including iNOS and cytokines [44, 45, 46]. In addition, flavonoids and phenolic compounds identified in *B. dalzielii* extracts [24] in our previous work have been widely reported to influence MAPK (ERK, JNK, and p38) and PI3K/Akt pathways [44, 45, 46], which are involved in keratinocyte migration, proliferation, and wound closure. The NO-lowering activity observed in the present study may therefore be attributed, at least in part, to the synergistic effects of these phytoconstituents.

The anti-inflammatory effects observed in this study are consistent with reports on other species within the genus *Boswellia*. Extracts and resin-derived compounds from *Boswellia serrata* and *Boswellia sacra* have demonstrated strong anti-inflammatory activities, including suppression of NO production, inhibition of pro-inflammatory cytokine release, and downregulation of leukotriene synthesis [47, 48, 49]. These pharmacological effects have largely been attributed to boswellic acids and related triterpenoids, which modulate inflammatory transcription factors and enzymatic pathways involved in inflammatory mediator production [38, 50, 51]. Although the phytochemical composition of *B. dalzielii* differs somewhat from more extensively studied species, the presence of structurally related phenolic and terpenoid compounds suggest comparable pharmacological potential. Reduction of NO production by plant extracts also correlates with improved wound healing, as excessive NO and oxidative stress are known to delay tissue repair [52]. The anti-inflammatory and antioxidant properties demonstrated by the *Boswellia* extracts in this study and related studies suggest that they may mitigate inflammatory burden while promoting tissue regeneration, aligning with traditional wound-healing applications.

Skin aging and impaired wound repair are strongly associated with degradation of extracellular matrix (ECM) components, particularly collagen, elastin, and hyaluronic acid. These structural macromolecules maintain skin elasticity, hydration, and tensile strength [53]. Enzymes such as collagenase, elastase, and hyaluronidase contribute to ECM breakdown and are frequently upregulated during inflammation, oxidative stress, and aging processes. Inhibition of these enzymes is therefore considered an important therapeutic strategy in anti-aging and skin-protective applications [53]. Natural plant extracts have attracted attention as potential sources of enzyme inhibitors due to their rich content of polyphenols, terpenoids, and other bioactive compounds capable of modulating ECM-degrading enzymes [54].

Plants belonging to the genus *Boswellia* have been widely used in traditional medicine for the treatment of inflammatory conditions and tissue injuries. Several species, including *Boswellia serrata* and *Boswellia frereana*, have demonstrated protective effects against tissue degradation, partly through antioxidant and anti-inflammatory mechanisms [55, 56, 57]. However, limited information exists regarding the ECM-protective enzyme inhibitory activities of *Boswellia dalzeilii*, especially concerning collagenase, hyaluronidase, and elastase inhibition. The present study therefore provides insight into the potential dermoprotective properties of aqueous leaf (BDL) and stem bark (BDSB) extracts of *B. dalzeilii*. The present findings demonstrate that aqueous extracts of *Boswellia dalzeilii* exhibit inhibitory activities against collagenase, hyaluronidase, and elastase, suggesting potential protective effects against extracellular matrix degradation. These enzymes play major roles in skin aging, inflammation, and impaired wound healing, making them important pharmacological targets.

The collagenase inhibition results revealed that both BDL and BDSB extracts suppressed enzyme activity in a time- and concentration-dependent manner. Progressive inhibition over 20 mins indicates sustained interaction between extract constituents and the enzyme system. At 50 µg/mL, BDL achieved slightly higher percentage inhibition than BDSB and produced activity comparable to the reference compound EGCG, with only a small difference in inhibition values. The IC_{50} values further confirmed the potency ranking, with EGCG showing the strongest inhibition, followed by BDL and BDSB. The ability of *B. dalzeilii* extracts to inhibit collagenase suggests the presence of phytochemicals capable of preventing collagen degradation and preserving dermal structural integrity [24].

Hyaluronidase inhibition assays demonstrated concentration-dependent enzyme suppression by both extracts, with BDSB displaying greater potency than BDL. The IC_{50} value of BDSB, although statistically different, approached that of tannic acid, a well-known hyaluronidase inhibitor, suggesting strong inhibitory activity. Nevertheless, tannic acid exhibited the highest inhibition. Hyaluronidase contributes to the degradation of hyaluronic acid, which is essential for maintaining skin hydration, viscoelasticity, and tissue repair. The inhibition of this enzyme by *B. dalzeilii* extracts may therefore help maintain extracellular hydration and structural stability. Elastase inhibition analysis also demonstrated strong enzyme suppression by the extracts, with BDSB showing superior activity compared to BDL. At the highest tested concentration, BDSB produced inhibition approaching that of quercetin and remaining lower than ursolic acid. The IC_{50} results reinforced this pattern, with BDSB displaying considerably higher potency than BDL and moderate activity relative to the reference compounds. Elastase degrades elastin fibers responsible for maintaining skin elasticity, and excessive elastase activity contributes to wrinkle formation and loss of skin resilience. The inhibitory activity of *B. dalzielii* extracts therefore suggests potential applications in preventing elastin degradation and maintaining dermal elasticity.

These observations are consistent with previous studies on other *Boswellia* species. Extracts of *Boswellia serrata* have been reported to protect connective tissues and suppress matrix metalloproteinases, including collagenase, elastase, and hyaluronidase enzymes, largely attributed to boswellic acids and related triterpenoids

[30]. Direct studies evaluating collagenase, elastase, and hyaluronidase enzymes inhibition by *B. dalzeilii* remain scarce in the literature, indicating that the present findings contribute new evidence supporting the ECM-protective potential of this species. The greater activity consistently observed with BDSB compared to BDL may be attributed to the variation in the concentrations of their bioactive constituents [24]. Studies on other *Boswellia* species have shown that bark and resin often contain abundant triterpenoids, including boswellic acids, which possess strong anti-inflammatory and tissue-protective activities [30, 51, 58]. In addition, monoterpenes and triterpenes identified in *Boswellia frereana*, such as α-pinene and epilupeol, have been shown to suppress inflammatory mediators and contribute to wound healing processes [34].

The inhibition of collagenase, hyaluronidase, and elastase collectively suggests that *B. dalzeilii* extracts may protect multiple ECM components simultaneously. Such multi-target enzyme inhibition is particularly valuable in dermoprotective and anti-aging formulations, as ECM degradation typically involves coordinated activity of several proteolytic enzymes. The observed enzyme inhibition may also complement previously reported anti-inflammatory and wound-healing properties of *Boswellia* species, further supporting their therapeutic potential in folklore.

The present study evaluated the antibacterial activity of aqueous leaf (BDL) and stem bark (BDSB) extracts of *Boswellia dalzielii* against two clinically important wound and skin pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The findings revealed marked species-dependent susceptibility, with both extracts producing substantially stronger growth inhibition against *S. aureus* than against *P. aeruginosa*. Such differential activity reflects known structural and physiological differences between Gram-positive and Gram-negative bacteria, which frequently influence the effectiveness of plant-derived antimicrobial compounds [59].

Both BDL and BDSB demonstrated pronounced antibacterial activity against *S. aureus*, with identical minimum inhibitory concentration (MIC) values of 0.31 mg/mL and complete suppression of bacterial proliferation across the incubation period. Comparable susceptibility of *Staphylococcus* species to *Boswellia* extracts has been reported in previous studies involving *B. dalzielii* and other members of the genus. Investigations of *Boswellia serrata* and *Boswellia sacra* have similarly documented strong inhibitory activity against *S. aureus* and methicillin-resistant *S. aureus*, which has been linked to the presence of bioactive triterpenoids, flavonoids, and phenolic compounds capable of disrupting cell membrane integrity and inhibiting essential bacterial metabolic processes [60, 61].

Phytochemical analyses conducted in several independent studies confirm that both leaf and stem bark of *B. dalzielii* contain classes of secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolics that have been implicated in antibacterial and anti-inflammatory activities in medicinal plants generally and within the genus *Boswellia* specifically [24, 62]. The congruence of antibacterial effects from different plant parts suggests that similar bioactive compounds are distributed across tissues, aligning with ethnobotanical evidence for the use of leaves, bark, and resins of *Boswellia* species in traditional infection remedies.

In contrast, *P. aeruginosa* exhibited limited susceptibility to both extracts, with measurable inhibition occurring only at the highest tested concentration (10 mg/mL). This reduced sensitivity aligns with the intrinsic resistance mechanisms of *P. aeruginosa*, including its impermeable outer membrane, multidrug efflux pump systems, and biofilm-forming ability, which collectively reduce the intracellular accumulation and effectiveness of antimicrobial agents [63, 64]. Similar resistance patterns have been reported for extracts of other *Boswellia* species as well as related Burseraceae plants, where Gram-negative bacteria frequently demonstrate lower susceptibility compared with Gram-positive organisms [62, 65, 66]. The selective antibacterial profile observed in this study therefore reflects a common pattern in plant-based antimicrobial investigations and reinforces the therapeutic relevance of *B. dalzielii* for infections predominantly associated with Gram-positive pathogens.

Importantly, the observation that OD_{600} values did not fall to near-zero under inhibitory conditions, attributed to extract precipitation, underscores a key technical consideration. OD_{600} can overestimate growth, and this is apparent when insoluble material contributes to turbidity. The pronounced antibacterial activity exhibited by both BDL and BDSB against *S. aureus* highlights the therapeutic potential of *B. dalzielii* as a source of botanical antimicrobial agents for wound and skin infections. Therefore, the species-specific selectivity observed here highlights the value of botanical antimicrobials as potential alternatives or adjuvants to conventional antibiotics, particularly for staphylococcal skin and wound infections.

5. Conclusion

This study demonstrates that aqueous leaf and stem bark extracts of *Boswellia dalzielii* possess significant dermatological therapeutic potential, exhibiting strong cytocompatibility with human keratinocytes. Both extracts enhanced keratinocyte migration, with stem bark extract showing superior activity. The extracts also suppressed nitric oxide and pro-inflammatory cytokines (IL-6 and IL-8) as well as inhibited extracellular matrix-degrading enzymes, indicating protective effects against skin inflammation, aging, and impaired wound healing. Additionally, selective antibacterial activity was observed, particularly against *Staphylococcus aureus*, supporting their relevance in managing Gram-positive skin infections. These findings provide scientific validation for the traditional use of *B. dalzielii* in wound and skin disorder management and highlight its potential for topical therapeutic development. Future work is planned to incorporate *in vivo* or *ex vivo* models to further validate these findings and support translational development.

List of Abbreviations

ATCC	American Type Culture Collection
BDL	*Boswellia dalzielii* Leaf extract
BDSB	*Boswellia dalzielii* Stem Bark extract
BHI	Brain Heart Infusion

CFU	Colony Forming Unit
ChC	Collagenase from *Clostridium histolyticum*
DMABA	p-Dimethylaminobenzaldehyde
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's Phosphate Buffered Saline
ECM	Extracellular Matrix
EGCG	Epigallocatechin gallate
ELISA	Enzyme-Linked Immunosorbent Assay
FALGPA	N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala
FBS	Fetal bovine serum; HaCaT: Human keratinocyte cell line
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IC_{50}	Half maximal inhibitory concentration
IFN-γ	Interferon gamma; IL-6: Interleukin-6
IL-8	Interleukin-8; iNOS: Inducible nitric oxide synthase
LPS	Lipopolysaccharide
MAPK	Mitogen-Activated Protein Kinase
MIC	Minimum Inhibitory Concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	Nuclear Factor kappa B
NO	Nitric oxide
NST	Non-stimulated
PVDF	Polyvinylidene fluoride
SC_{50}	Half maximal scavenging concentration
ST	Stimulated
Suc-AAA-pNA	N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide
TNF-α	Tumor necrosis factor alpha
TX-100	Triton X-100
UHPLC-DAD-MS	Ultra-High-Performance Liquid Chromatography with Diode Array Detection and Mass Spectrometry
UV	Ultraviolet

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