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In vitro Antioxidant, Anti-Inflammatory, and Wound Healing Properties of *Aloe Vera* Infused with Canola Oil for Possible Application in Skin Cosmetics

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Abstract

This research investigates the antioxidant, anti-inflammatory, and wound-healing properties of *Aloe vera* oil, along with its cytotoxic effects on normal human dermal fibroblasts (NHDF), particularly in the context of its potential use as a natural antioxidant in skincare formulations. *A. vera* oil was extracted using an infusion method with canola oil, and its properties were thorough characterized. The concentration of aloin and cycloartenol in the *A. vera* oil were measured using high-performance liquid chromatography (HPLC). The resulted indicated that *A. vera* oil exhibited no cytotoxicity towards NHDF cells. Additionally, the oil demonstrated antioxidant activity, as evidenced by its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, and its reducing potential assessed through free radical scavenging activity (FRAP) assays. Regarding its anti-inflammatory properties, the *A. vera* oil at a specific concentration showed no cytotoxic effects on RAW 264.7 macrophage cells. Notably, its potent anti-inflammatory effects significantly ($p \leq 0.05$) reduced nitric oxide (NO) productions in RAW 264.7 macrophage cells stimulated by lipopolysaccharide (LPS), compared to the LPS-treated cells group. Moreover, NHDF cells treated with *A. vera* oil exhibited a statistically significant ($p \leq 0.05$) improvement in wound closure area in a scratch wound assay, indicating enhanced cell migration. Collectively, these findings highlight the antioxidant, anti-inflammatory, and wound-healing properties of *A. vera* oil, underscoring its potential as an eco-friendly and beneficial ingredient for cosmetic skincare products.

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Introduction

Skincare products are designed to nourish the skin while addressing and correcting various imperfections. They play a vital role in preserving the skin's moisture levels, contributing to a soft, radiant, and wrinkle-free complexion. Suitable for individuals of all genders, these products are primarily aimed at maintaining optimal skin hydration. Commonly referred to as moisturizers, they typically contain ingredients that hydrate, soften, and enhance the smoothness of the skin's surface. Their composition often includes emollients, humectants, occlusive agents, and natural moisturizing factors (NMFs) (Surber & Kottner, 2016). Humectants, including hygroscopic compounds like propylene glycol and glycerin, facilitate water absorption by the stratum corneum. They draw moisture from both the dermis and the surrounding humid environment into the epidermis. In contrast, occlusive agents, such as beeswax and petroleum, are oil-based substances that preserve the skin's moisture content by forming a hydrophobic barrier on the skin's surface, thereby reducing transepidermal water loss. Emollients, primarily composed of lipids and their derivatives such as fatty acids (e.g., palm oil, and coconut oil) and cholesterol fill the gaps between intercorneocyte clusters. This action is widely utilized in cosmetic formulations or topical pharmaceuticals to significantly improve skin hydration, smoothness, softness, and flexibility (Purnamawati et al., 2017). Currently, both natural and synthetic anti-inflammatory agents, such as *A. vera*, bisabolol, and glycyrrhetinic acid, are combined with emollients or humectants to enhance barrier repair and effectively manage dry skin (Varothai, Nitayavardhana, & Kulthanan, 2013; Dal'Belo, Gaspar, & Maia Campos, 2006). Natural oils are now commonly used as moisturizer ingredients due to their hydrating and protective effects on the skin. For instance, Zhou et al. (2024) demonstrated that oil derived from *C. oleifera* seeds exhibits remarkable moisture retention capabilities, possesses anti-inflammatory properties, and shows effective free radical scavenging activity. Furthermore, incorporating *C. oleifera* seed oil into emulsions significantly enhances both the antioxidant and moisturizing properties of the formulation compared to emulsions lacking this oil.

In recent years, botanical ingredients have become a primary source of materials for the cosmetics and pharmaceutical industries, driving increased interest in plant-derived products with beneficial properties for skincare. Their key advantages include being gentle yet effective, safe, non-toxic, and devoid of adverse effects. Cosmetics enriched with bioactive compounds are particularly well-suited to address specific skin needs and offer greater environmentally sustainability compared to traditional cosmetic products. A notable category of natural ingredients frequently utilized in cosmetics is plant extracts, which serve as a rich reservoir of biologically active substances that significantly benefit human skin. These extracts exhibit a wide range of properties, including medicinal benefits for various skin conditions, such as inflammatory disorders like acne, psoriasis, and atopic dermatitis. They also offer diverse skincare applications including antioxidants, antibacterial, astringent, moisturizing, regenerating, cleansing, smoothing, and lightening effects. (Michalak, 2022). Notably, plant-based antioxidants such as extracts from green tea, *Aloe vera*, and chamomile, are increasingly incorporated into topic formulations. (Arulselvan et al., 2016). *A. vera* (L.) Burm. f. (*Aloe barbadensis* Miller), a perennial species in the Liliaceae family, is well-known for its traditional medicinal applications, particularly in managing skin ailments, burns, inflammation, acne, and fungal infections. The gel-like substance within *A. vera* is rich in bioactive compounds, including aloin, glycoproteins, anthraquinones, glycosides and plant sterols. These constituents exhibit a variety of biological activities, such tumor growth inhibition, promotion of wound healing, antimicrobial and antifungal effects, anti-inflammatory properties, and antioxidant capabilities (Sánchez et al., 2020). Furthermore, a solution of *A. vera* gel has been shown to promote cell proliferation and migration in normal human primary skin fibroblasts and keratinocytes cultured in growth media (Teplicki et al., 2018). Moreover, Hekmatpou et al. (2019) established that *A. vera* can retain skin moisture and integrity. It has been used to prevent skin ulcers and treat various conditions, including burn injuries, postoperative wounds, fissured nipples, genital herpes, psoriasis, and chronic wounds such as pressure ulcers. *Aloe* sterol, a key compound found in *A. vera* gel, exhibits distinct therapeutic properties. It is categorized into two main classes of compounds: 1) lophenol, which includes lophenol, 24-methyl-lophenol, and 24-ethyl-lophenol, and 2) cycloartane, comprising cycloartanol and 24-methylene-cycloartanol (Tanaka et al., 2006). Tanaka et al. (2015) indicated that participants receiving *Aloe*

sterol experienced enhanced skin moisture, elasticity, and collagen levels compared to those in the placebo group. Additionally, *Aloe* sterol significantly reduced the total protein expression of MMP-2 and MMP-9 (including both active and pro forms) in mice exposed to UVB radiation. (Saito et al., 2016). A validated high-performance liquid chromatography (HPLC) method was employed to quantify the content of *Aloe* sterol in samples derived from *A. vera* gel extracts. The study included *A. vera* gel extracted using a chloroform/methanol mixture and *A. vera* gel prepared by dispersing gel powder in an edible oil, followed by extraction using supercritical CO_2. The findings indicated that *A. vera* gel oil predominantly contained cycloartanol and 24-methylene-cycloartanol with no significant differences observed when compared to *A. vera* gel extracted with organic solvents (Nabeshima et al., 2022). Additionally, *A. vera* gel prepared by dispersing gel powder in an edible oil, followed by extraction using supercritical CO_2 exhibited safety profiles in both in vitro and in vivo studies (Tanaka et al., 2012). Research conducted by Kaminaka et al. (2020) indicated that the supplementation of *Aloe* sterols enhanced skin hydration by facilitating the function of the skin barrier and increasing dermal collagen synthesis, thereby supporting the preservation of healthy skin.

In the field of cosmetics, *A. vera* is predominantly available in five primary forms: 1) liquid concentrate, 2) powder, 3) oil, 4) stabilized gel, and 5) pulp (Javed & Rahman, 2014). However, the powdered variant of *A. vera*, however, poses certain challenges, notably its vulnerability to oxidation when exposed to air, which compromises its biological effectiveness, as well as increased production costs. As a result, *A. vera* gel and juice are more commonly employed, despite their own drawbacks, such as the risk of microbial contamination and shorter shelf life. A combination of concentrated ozone and *A. vera* oil, referred to as ozonated *A. vera* oil, demonstrated efficacy in enhancing the healing response of full-thickness wounds. This treatment resulted in an increased proliferation of fibroblasts and a thickening of collagen, which subsequently expedited the wound healing process in Sprague-Dawley rats (Taqwim Hidayat et al., 2021). The tenets of green chemistry suggest that vegetable oils may offer a competitive advantage, particularly when considering economic factors, food safety, and environmental sustainability (Yara-Varón et al., 2017). These oils are abundant in bioactive compounds, with a notable relationship observed between the solubilization of both non-polar and polar bioactive substances and the roles of fatty acids, lipid classes, and other minor components (Li et al., 2014). Common edible oils such as soybean, olive, sunflower, corn, grapeseed, and canola oils demonstrate the capability to extract carotenoids, antioxidants (including astaxanthin), as well as aromatic and phenolic compounds (Yara-Varón et al., 2017). Among edible oils, canola oil distinguishes itself as a significant source of various bioactive compounds, ranking as the second most produced edible oil worldwide after soybean oil (Portillo-López et al., 2021). This investigation focused on the biological activities of dried *A. vera* powder extracted using canola oil, evaluating its antioxidative, anti-inflammatory, wound healing, and cytotoxic effects on NHDF cells. Freeze-dried *A. vera* pulp was infused with canola oil, and the active components of *A. vera* oil, particularly cycloartanol and aloin, were analyzed. To integrate *A. vera* oil into skincare formulations, its in vitro cytotoxic effects on NHDF cells, along with its antioxidant, anti-inflammatory, and wound healing properties, were thoroughly examined. This study represents the first comprehensive exploration of this subject.

Materials and Methods

1. Materials and chemicals

Fresh, healthy, and mature leaves of *A. vera* was harvested from Homkajorn farm, Suan Dusit University, Suphanburi campus, Suphanburi province, Thailand in September 2023. Aloin, cycloartenol, Trolox and 2,4,6-tri (Z2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma-Aldrich, USA. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Alfa, USA, while ethanol was sourced from Merck, Germany. Formic acid, acetonitrile, water, and dimethyl sulfoxide (DMSO) were acquired from Fisher, England. Hydrochloric acid and ferric chloride were supplied by Qrec, New Zealand. Penicillin streptomycin, fetal bovine serum (FBS) and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Gibco, USA. The normal human dermal fibroblast (NHDF) cell line was obtained from ATCC, USA. RAW 264.7 macrophage cell line was acquired from Elabscience Bionovation, USA. The nitric oxide assay kit was purchased from Thermo Fisher, Austria, while sterile water was provided by A.N.B. Laboratories, Thailand. Lastly, the Griess reagent kit was obtained from Invitrogen, Austria.

2. Production of *A. vera* oil

A yellow liquid was obtained by severing the base of the *A. vera* leaves and allowing them to drain vertically for 1 h. (Di Scala et al., 2013). The leaves were then washed with tap water to remove the dust and impurities, followed by air drying at room temperature for 4 h. The *A. vera* peel was discarded, and the pulp was collected. The pulp was cut into small pieces and stored at -20°C for 24 h. Drying of *A. vera* was performed using a freeze-drying method at -50°C for 72 h. after which the material was ground into a fine powder. Heavy metal content in the dried *A. vera* powder, including arsenic, cadmium, lead, and mercury was analyzed using the In-house method SOP 06-02 179 AAS technique. The dried *A. vera* powder was then infused with canola oil at a 1:5 weight ratio and stored in the dark at room temperature for 7 days.

3. Determination of physicochemical properties

The physicochemical properties of *A. vera* oil were evaluated based on physical appearance, L* a* b* color, odor, and pH value. Physical appearance and color were assessed through visual observation while odor was evaluated by directly smelling paper strips containing the oil. The L* a* b* color value was measured using a Chroma meter (CR-400, Centasia, Konica Minolta, Thailand). The pH value was measured using pH meter.

4. Quantification of aloin and cycloartenol

The levels of aloin and cycloartenol in *A. vera* oil were analyzed using a validated high-performance liquid chromatography (HPLC) technique, as reported by Nabeshima et al. (2022) with modifications. The separation process employed a C18 column (VertiSep™ GES, 4.6x150 mm, 5µm particle size), with the mobile phases consisting of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile), following a gradient elution method. The flow rate was maintained at 1.0 mL/min, and the column temperature was controlled at 40°C. A photodiode array detector was utilized, operating within a wavelength range of 190-400 nm, with a specific focus at a wavelength of 357 nm. The injection volume for both the sample and the reference standards (aloin and cycloartenol) was set.

5. Cytotoxicity assay

A cytotoxicity assay was conducted to evaluate the cell viability of the cultured fibroblast cells using MTT assay as previously reported by Ala et al. (2018), with slight modifications. Normal human dermal fibroblast (NHDF) cells was used as the model to study cytotoxicity. The NHDF cells (ATCC#PCS-201-010) were subjected to trypsinization and then plated at a density of approximately $1x10^4$ cells per well in a 96-well plate. They were cultured in complete growth medium consisting of DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1.0% (v/v) penicillin/streptomycin. The cells were incubated at 37°C in a 5%CO_2 environment for 24 h. After this period, the culture medium was discarded, and the cells were rinsed with sterile phosphate-buffered saline (PBS). The NHDF cells were treated with varying concentrations of *A. vera* oil (5, 10, 25, 50, 100, and 250 µg/mL) in comparison to a control group. After a 24-h. incubation period, the media containing the samples was carefully removed, and the cells were washed with sterile PBS. The NHDF cells were incubated with MTT solution at 37°C in the dark for 2 h. Following incubation, the medium was discarded, and the resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO). Absorbance was measured at a wavelength of 570 nm using a microplate reader (Synergy H1, BioTek, USA) Cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = (AS/AC) \times 100 \quad [1]$$

Where AS is the absorbance of the sample being tested, while AC is the absorbance of the control, consisting of untreated cells.

6. *In vitro* antioxidant assays

6.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effect of *A. vera* oil was assessed following the method reported by Yakaew et al. (2020) with slight modifications. Specifically, 150 µL of a DPPH ethanolic solution (0.2 mM) was mixed with 75 µL of *A. vera* oil solution. The mixture was incubated at room temperature for 30 min in a dark environment. After the incubation, the absorbance was measured at 515 nm using a microplate reader (Biochrom, EZ Read 2000, England). This assay was performed in triplicate and compared to the positive control, Trolox. The radical scavenging activity was expressed as a percentage of DPPH decolorization using the specified equation.

$$\text{DPPH radical scavenging activity (\%)} = (A_B\text{-}A_S/A_B) \times 100 \quad [2]$$

Where A_S represents the absorbance of DPPH in the presence of the tested sample, and AB denotes the absorbance of DPPH in the absence of the tested sample.

The concentration that resulted in 50% inhibition (IC_{50}) was determined from the graph showing the percentage of inhibition relative to the sample concentration. The assay was performed in triplicate.

6.2 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power of *A. vera* oil was determined using the (FRAP) assay as previously described by Sulaiman et al. (2011) with slight modifications. The FRAP reagent was prepared by combining acetate buffer (300 mM, pH 3.6), a 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$ in a volume ratio of 10:1:1 (v/v/v). Subsequently, 190 µL of the FRAP reagent was mixed with 10 µL of *A. vera* oil solution at a concentration of 1 mg/mL. The mixture was incubated at 37°C for 30 min in a dark environment. After incubation, the absorbance was measured at 593 nm with a microplate reader (Biochrom, EZ Read 2000, England). The results were expressed as mg of Trolox equivalent per g of extract, based on the calibration curve. This assay was conducted in triplicate.

7. Anti-inflammatory activity

7.1 Cell Culture

The murine macrophage cell line RAW 264.7 (EP-CL-0190, Elabscience, USA) was seeded into 96 well-plates at a density of $1x10^4$ cells/well in RPMI-1640 medium (GIBCO, Paisley, UK) supplemented with 10% (v/v) fetal bovine serum (GIBCO, Paisley, UK) and 1.0% (v/v) penicillin/streptomycin (GIBCO, Paisley, UK). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h.

7.2 Cytotoxicity

A cytotoxicity assay was also conducted to evaluate the cell viability of the cultured RAW 264.7 macrophages cells using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, following a previously described method with some modifications (Lim et al., 2018). RAW 264.7 macrophages were seeded at a density of $1x10^4$ cells/well were cultured for 24 h. The cells were treated with *A. vera* oil at various concentrations ranging from 0 to 250 µg/ mL alongside a control group. DMSO was used as the vehicle control, and the final concentration did not exceed 0.2% (v/v). After an additional 24-h incubation period, the spent medium was then removed. Subsequently, 20 µL of MTT reagent was added to each well, and the plate was incubated at 37°C in a CO_2 incubator for two hr. The absorbance of each well was measured at 570 nm using a microplate reader (Synergy H1, BioTek, USA).

7.3 Inhibition of nitric oxide (NO) production

The anti-inflammatory properties of *A. vera* oil were assessed using the RAW 264.7 murine macrophage cell line assay, following a modified protocol based on previous research (Lim et al., 2018). RAW 264.7 cells were cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum and 1.0% (v/v) penicillin/streptomycin. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. Subsequently, the macrophages were plated in a 96-well culture dish at a density of 5×10^4 cells per well and incubated for an additional 24 h. Following this, the cells were pre-treated with 100 µL of *A. vera* oil at varying concentrations (0-25 µg/mL) for 30 min. They were then exposed to 100 µL of LPS (1.0 µg/mL) and incubated at 37°C in a 5% CO_2 environment for 24 h. The secretion of nitric oxide (NO) in the culture supernatant was measured using the Griess reagent kit and assessed through the Griess assay, with an incubation period of 30 min at room temperature. The NO concentration was quantified at 540 nm using a microplate reader (CLARIO star, BMG Labtech, DKSH Technology Limited, Thailand). Results were expressed as a percentage of nitric oxide relative to the DMSO control in LPS-treated cells, with indomethacin (50 µM) serving as the positive control.

8. In vitro wound healing scratch assay

8.1 Human skin fibroblast cell culture

Normal human dermal fibroblast (NHDF) cells were grown in DMEM enriched with 10% (v/v) fetal bovine serum (FBS) and 1.0% (v/v) penicillin/streptomycin. The cells were maintained in a humidified atmosphere at 37°C with 5% CO_2. The growth medium was replenished every 48 to 72 h. NHDF cells were sub-cultured using a 0.25% trypsin/EDTA solution upon reaching 70% to 80% confluency.

8.2 Wound healing scratch assay

The effect of *A. vera* oil on wound healing was evaluated using a modified scratch wound technique, as described by Radstake et al. (2023). Normal human dermal fibroblast (NHDF) cells were plated in a 48-well culture dish at a density of 5×10^4 cells per well and cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1.0% (v/v) penicillin/streptomycin. The cells were maintained in a humidified environment at 37°C with 5% CO_2. After incubation, a scratch was created in the open wound area using a 200 µL pipette tip. The cells were then rinsed with sterile phosphate-buffered saline (PBS) at pH 7.4 to remove

debris. Baseline images were captured immediately after the wound was created to document the initial state of the wound.

For the migration assay, NHDF cells were cultured in DMEM enriched with 1.0% (v/v) antibiotics and 0.5 mL per well of *A. vera* oil at concentrations of 5 and 10 µg/mL. The cells were maintained in a humidified environment at 37°C with 5% CO_2 for a duration of 24 h. A positive control was established using DMEM supplemented with 10% (v/v) FBS and 1.0% (v/v) penicillin/streptomycin, while a negative control consisted of DMEM and 1.0% (v/v) antibiotics. Gap closure data were expressed as a percentage of the remaining area relative to the initial scratch area (T0) and compared to *Aloe* vera oil group after 24 h of incubation (T24). Following the incubation period, the insert was carefully removed. The cells were fixed with 4% (v/v) paraformaldehyde for one hr, followed by staining with 0.5% crystal violet solution for 30 min Excess dye was removed by washing the insert briefly with PBS.

8.3 Microscopy

NHDF cells were imaged 24 h after wounding. Images were captured using the phase contrast mode and brightfield mode of inverted microscope (Primo Vert, ZEISS, Carl Zeiss Micro Imaging GmbH, Germany) at 40× magnification.

9. Statistical Analysis

Data were expressed as mean values±standard deviation (SD) using Microsoft Excel 2013. Statistical analysis of the data was analyzed by performing a one-way analysis of variance (ANOVA) and Duncan's post-hoc tests using SPSS program (SPSS ver. 22.0 for Windows, SPSS Inc., Chicago, IL, USA) and the statistical significance was $p \leq 0.05$.

Results and discussion

1. Physicochemical characterizations of *A. vera* oil

The freeze-dried *A. vera* gel appears as a fine white powder (Fig. 1A). Analysis confirmed that this *A. vera* powder was free from contamination by the four heavy metals; arsenic, cadmium, lead, and mercury, which are prohibited in raw materials intended for cosmetic production. These results indicate that the *A. vera* gel studied is safe for use as a raw material cosmetic product development. The freeze-dried *A. vera* powder was then extracted using an infusion method with canola oil for 7 days at room temperature (Fig 1B). The physicochemical properties of the extracted *A. vera* oil were then evaluated. The results showed that *A. vera* oil appeared as a clear yellow liquid with no rancid odor and its pH value was measured at 5.0 (Fig. 1C). The color of *A. vera* oil displayed a more intense green and yellow hue compared to the synthetic and semi-synthetic emollients, as reported by Ogorzałek et al. (2024). The acidic pH of *A. vera* was attributed to the presence of fatty acid constituents in canola oil. These findings suggest that *A. vera* oil is well-suited for incorporation into topical skin formulations, particularly when the skin's pH is within the range of 4 to 6 (Lukić et al., 2021).

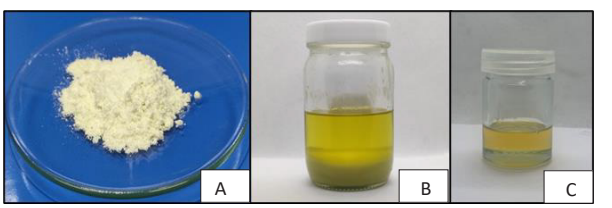

Fig. 1 The visual characteristics of (A) freeze-dried *A. vera* powder, (B) *A.vera* powder infused with canola oil and (C) *A. vera* oil

2. Quantification of aloin and cycloartanol in *A. vera* oil

The HPLC chromatograms of aloin and cycloartanol standards, as well as *A. vera* oil are presented in Fig. 2A and 2B, respectively. The cycloartanol content in *A. vera* oil was measured at 800.95 µg/g extract with no detectable amounts of aloin, as shown in Fig. 2B. This observation aligns with previous studies reporting that cycloartenol is found in both the gel and peel of *A. vera* (Tanaka et al., 2006). In contrast, aloin is predominantly located in the peel and latex of *A. vera* leaves. Additionally, Tanaka et al. (2015) investigated the presence of *Aloe* sterols in *A. vera* gel powder using liquid chromatography-tandem mass spectrometry. The findings indicated that a sample of approximately 0.5 g of *A. vera* gel powder contained around 40 µg of *Aloe* sterols, specifically comprising 11 µg of lophenol, 13 µg of 24-methyl-lophenol, 5 µg of 24-ethyl-lophenol, 6 µg of cycloartanol, and 8 µg of 24-methylene-cycloartanol. Furthermore, the research highlighted the beneficial properties of *Aloe* sterols, such as enhancing skin hydration, promoting the production of hyaluronic acid (HA), and stimulating collagen synthesis. The *Aloe* sterols were quantified in raw materials, including *A. vera* gel powder extracted using chloroform/methanol and *A. vera* gel oil obtained through supercritical carbon dioxide (CO_2) extraction. The content ratios of these sterols were calculated and

analyzed using a validated high-performance liquid chromatography (HPLC) method. The analysis revealed that the content ratios of the five sterols in *A. vera* gel powder were 9%, 20%, 11%, 32%, and 28%. Meanwhile the corresponding ratios in *A. vera* gel oil extraction were 11%, 20%, 10%, 30%, and 29% for lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol, respectively. These findings align with studies and suggest that the cycloartanol content in *A. vera* oil is greater than what has been previously reported.

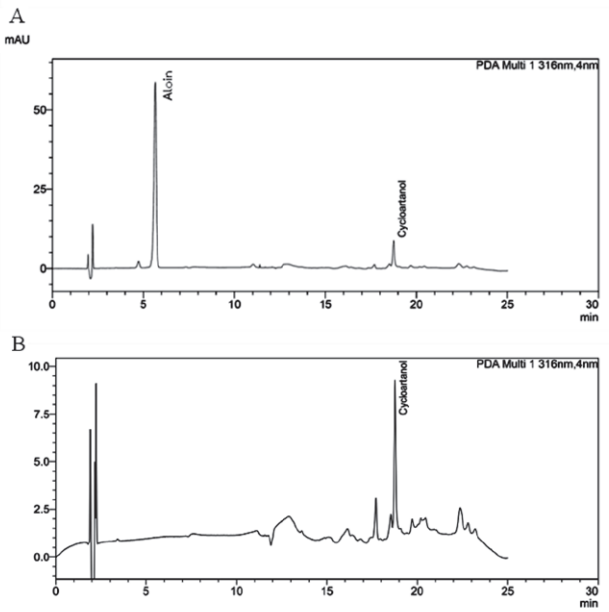

Fig. 2 HPLC chromatograms of (A) aloin and cycloartanol and (B) *A. vera* oil (1.0 mg/mL)

3. Cytotoxicity to normal human dermal fibroblasts

One of the key criteria for assessing the cytotoxicity test of *A. vera* oil is cell viability. The cytotoxic effect of the *A. vera* oil on normal human dermal fibroblast (NHDF) cells was evaluated using MTT assay. The results are expressed as percentage of cell viability and the corresponding concentrations of *A. vera* oil are shown in Fig. 3. The various concentrations of *A. vera* oil (ranging from 5.0 to 250 µg/mL) did not affect the viability of normal human skin fibroblasts after a 24-h incubation period, with cell viability remaining above 70% (Che Zain et al., 2020). The investigation into the toxicity of *A. vera* extract on NHDF cells, utilizing the MTT assay, revealed that concentrations ranging from 5 to 250 µg/mL yielded a cell survival rate exceeding 70%. This indicates that the extract is safe for incorporation into cosmetic formulations. Such findings provide as an initial guideline for the development of product formulations, particularly in determining the appropriate concentration of the extract to be utilized. According to Hattingh et al. (2023) and Lopez-Garcia et al. (2014), a cell survival rate above 70% classifies the tested substance as non-cytotoxic. Conversely, survival rates between 60% and 80% reflect slight cytotoxicity, while rates between 40% and 60% indicate moderate cytotoxicity. A survival rate below 40% is indicative of strong cytotoxicity. The research findings suggest that the concentrations of *A. vera* oil examined were non-toxic to normal human skin fibroblasts in vitro. Furthermore, *Aloe* sterols did not significantly affect the viability of human dermal fibroblasts (Tanaka et al., 2015).

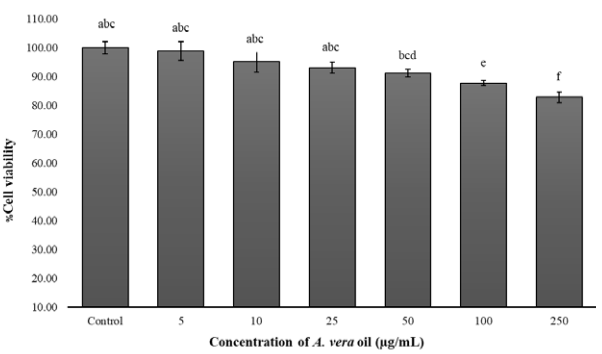

Fig. 3 The percentages of NHDF cell viability after 24-h exposure to *A. vera* oil. Each bar illustrates the mean±standard deviation (SD) from triplicate samples (n=3). Distinct subscript letters on the chart for each concentration of *A. vera* oil indicate significant differences compared to the control cells (untreated, 0 µg/mL) at a significance level of $p \leq 0.05$

4. Antioxidant activities of *A. vera* oil

The antioxidant properties of *A. vera* oil were evaluated using the DPPH scavenging method, while its reducing capacity was assessed through free radical scavenging activity, as determined by the FRAP assay. The DPPH assay measures a compound's ability to function as a free radical scavenger or a hydrogen donor. The results demonstrated that the DPPH radical scavenging effect was dose-dependent for both Trolox and *A. vera* oil. At a concentration of 60 mg/mL, *A. vera* oil exhibited a scavenging activity of 52.35±0.99%, whereas Trolox showed a significantly higher scavenging activity of 92.36±0.21% at a concentration of 1.0 mg/mL. Antioxidant effectiveness was quantified using the IC_{50} value, representing the concentration required for

50% inhibition. The findings revealed that *A. vera* oil possesses antioxidant properties, with an IC_{50} value of 56.75±0.81 mg/mL. In contrast, Trolox demonstrated superior antioxidant activity, with an IC_{50} of 0.08±0.001 mg/mL. The lower scavenging activity of *A. vera* oil compared to Trolox can be attributed to its crude state, which inherently reduces its free radical scavenging capacity compared to the purified compound.

The antioxidant power of *A. vera* oil was evaluated using the FRAP assay, which assesses the reducing capacity of an antioxidant through its reaction with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex. This reaction produces a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex. The results were expressed as milligrams of Trolox equivalent per gram of sample (mg TE/g), based on the Trolox calibration curve equation: ($y=0.7021x+0.1764$, $R^2=0.9998$). The analysis showed that the *A. vera* oil demonstrated FRAP activity, quantified at 285.47±5.46 mgTE/g of extract.

Previous studies have demonstrated that crude methanolic and ethanol extracts of *A. vera* possess potential antioxidant properties (Sánchez et al., 2020). The antioxidant effects of *A. vera* are partly attributed to the presence of anthraquinones, which exhibit the ability to scavenge peroxyl radicals and possess reducing capabilities (Sun et al., 2017). Additionally, research highlights that phytosterols, such as fucosterol, stigmasterol, and cycloartenol, possess various biological activities, particularly antioxidant and anti-inflammatory effects (Vezza et al., 2020; Truong et al., 2023). Consequently, the antioxidant properties of *A. vera* oil may be influenced by the presence of phytosterols within the oil.

5. Anti-inflammatory activity

5.1 Measurement of cell viability

Cytotoxicity was evaluated in RAW264.7 cells treated with *A. vera* oil using the MTT assay, with concentrations ranging from 5.0 to 250 µg/mL, as illustrated in Fig. 4. Cell viability was assessed relative to the untreated control group, which was set at 100% viability. The findings indicate that *A. vera* oil reduced cell viability in a concentration-dependent manner. Notably, significant decreases in cell viability ($p<0.05$) were observed at concentrations between 50 and 250 µg/mL, compared to the control group. In contrast, lower concentrations of *A. vera* oil (5-25 µg/mL), maintained cell viability above 70%, indicating a lack of cytotoxic effects at these levels. Based on these findings, lower concentrations (5, 10, and 25 µg/mL) were selected for further studies on the reduction of nitric oxide (NO).

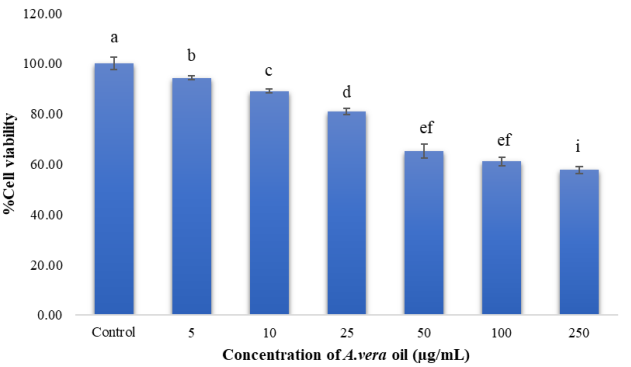

Fig 4. The percentages of RAW264.7 cell viability after 24-hr exposure to *A. vera* oil. Each bar represents mean ± standard deviation (SD) from triplicate samples (n=3). Different subscript letters on the chart for each extract indicate statistically significant differences when compared with control cells (untreated) at $p\leq0.05$.

5.2 Measurement of nitric oxide (NO) production

Macrophages play a vital role in modulating immune responses and managing inflammation. However, excessive production of nitric oxide (NO) can lead to inflammatory disorders and oxidative damage to cells and tissues, potentially contributing to genetic mutations and nerve injury (Sharma, & Al-Omran, 2007). Consequently, suppressing NO production can help prevent inflammation-related diseases. In this study, NO was generated by activating the mouse macrophages cell line RAW264.7 with lipopolysaccharide (LPS), and its levels were measured using Griess reagent in the form of nitrite ions (NO_2^-) in the culture medium. The results are presented in Fig. 5. The effects of *A. vera* oil on the production of proinflammatory mediators were evaluated by measuring NO levels in LPS-stimulated RAW 264.7 cells. The proportion of nitric oxide (NO) generated in the group of cells treated with LPS (control media + LPS) was recorded at 102.19±2.31%. Indomethacin, used as a positive control, significantly reduced NO production ($p<0.05$) compared to the LPS-treated cell group with a value of 63.86±2.13%. Similarly, each concentration of *A. vera* oil (5, 10, and 25 µg/mL) also demonstrated. The application of *A. vera* oil at concentrations of 5, 10, and 25 µg/mL resulted in reductions in nitric oxide (NO) production of 96.35±0.91%, 98.24±0.20%, and 97.24±0.47%, respectively. These findings demonstrate a statistically significant decrease ($p\leq0.05$) in NO production compared to the control group, which consisted of control media supplemented with lipopolysaccharide (LPS). However, the inhibitory effect of *A. vera* oil on NO production was lower than that observed with indomethacin, which served as a positive

control. Our research findings were consistent with previous studies indicating that the production of nitric oxide at the site of inflammation was noticeably suppressed by the *A. vera* extract (Wang et al., 2023). Furthermore, the alcoholic extract of *A. vera* has been shown to reduce inflammatory markers, such TNF-α, TGF-β, and IL-6, in rats subjected to a high-fat diet (Yazdani, Hossini, & Edalatmanesh, 2022). These observations suggest that the anti-inflammatory properties of *A. vera* may be attributed to compounds such as aloin, aloe-emodin, and acemannan (Sánchez et al., 2020). Additionally, phytosterols such as fucosterol, stigmasterol, and cycloartanol are known to exhibit anti-inflammatory effects (Vezza et al., 2020; Truong et al., 2023). This suggests that *A. vera* oil possesses anti-inflammatory properties, with compounds present the potentially contributing to the reduction of nitric oxide (NO) production.

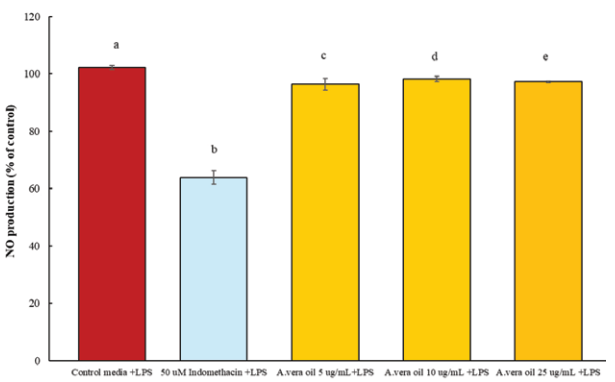

Fig. 5 Nitric oxide production in RAW264.7 macrophage cells after treatment with LPS, indomethacin, and *A. vera* oil at concentrations of 5.0 µg/mL, 10 µg/mL, and 25 µg/mL for 24 h. Each bar represents the mean±standard deviation (SD) from triplicate measurements (n = 3). Significant differences ($p \leq 0.05$) compared to the control group are indicated by varying bar patterns

6. *In vitro* wound healing scratch assay

The scratch wound assay is a widely utilized method for evaluating cell migration. The results obtained indicated that *A. vera* oil at concentrations of 5.0 µg/mL (59.76±1.32%) and 10 µg/mL (55.58±0.76%) significantly enhanced the wound closure area compared to the control group (42.28±3.27%) after 24 h of treatment. These differences were statistically significant ($p<0.05$), and are shown in Fig. 6.

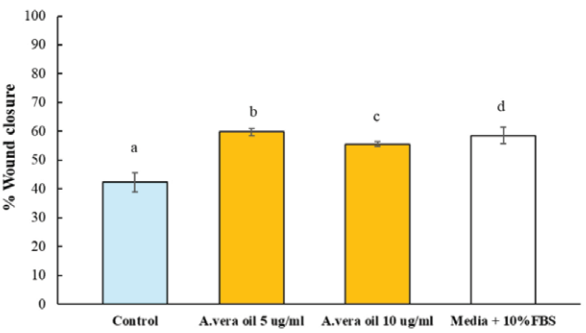

Fig. 6 Percentage of wound confluence indicating the rate of NHDF wound closure at 24 h compared to untreated cells (control). Each bar represents mean ± standard deviation (SD) from triplicate samples (n=3). The different bars on the chart for each sample indicate statistically significantly differences($p \leq 0.05$) compared to the control group

Fig. 7 With and without the application of *A. vera* oil at baseline (0 hr) and after 24 hr, morphological analysis revealed that both concentrations of *A. vera* oil significantly enhanced NHDF migration and proliferation. The treated wound area exhibited greater closure compared to the control group, suggesting that *A. vera* oil possesses wound-healing properties. These are consistent with previous studies, which attribute the wound-healing effects of *A. vera* to its impact on fibroblast cells (Shafaie et al., 2020). Negahdari et al. (2017) revealed that the ethanolic extract of *A. vera* gel promotes the migration and proliferation of fibroblast cells. Similarly, Teplicki et al. (2018) reported that *A. vera* significantly enhanced both the proliferation and migration of fibroblasts and keratinocytes. Moreover, earlier studies have identified acemannan and β-sitosterol, key components found in *A. vera* gel, as valuable to the wound healing process (Moon et al., 1999; Liu et al., 2006).

Conclusion

In this study, *A. vera* oil, extracted through infusion with canola oil, was identified to contain cycloartenol as its primary component, based on HPLC analysis. The findings revealed that *A. vera* oil exhibited no cytotoxic effects on normal human dermal fibroblasts (NHDF). Additionally, *A. vera* oil demonstrated significant in vitro antioxidant properties, as evidenced by DPPH scavenging activity and reducing power. Its anti-inflammatory effects were also highlighted through the inhibition of nitric oxide (NO) production, an inflammatory mediator, making it a promising

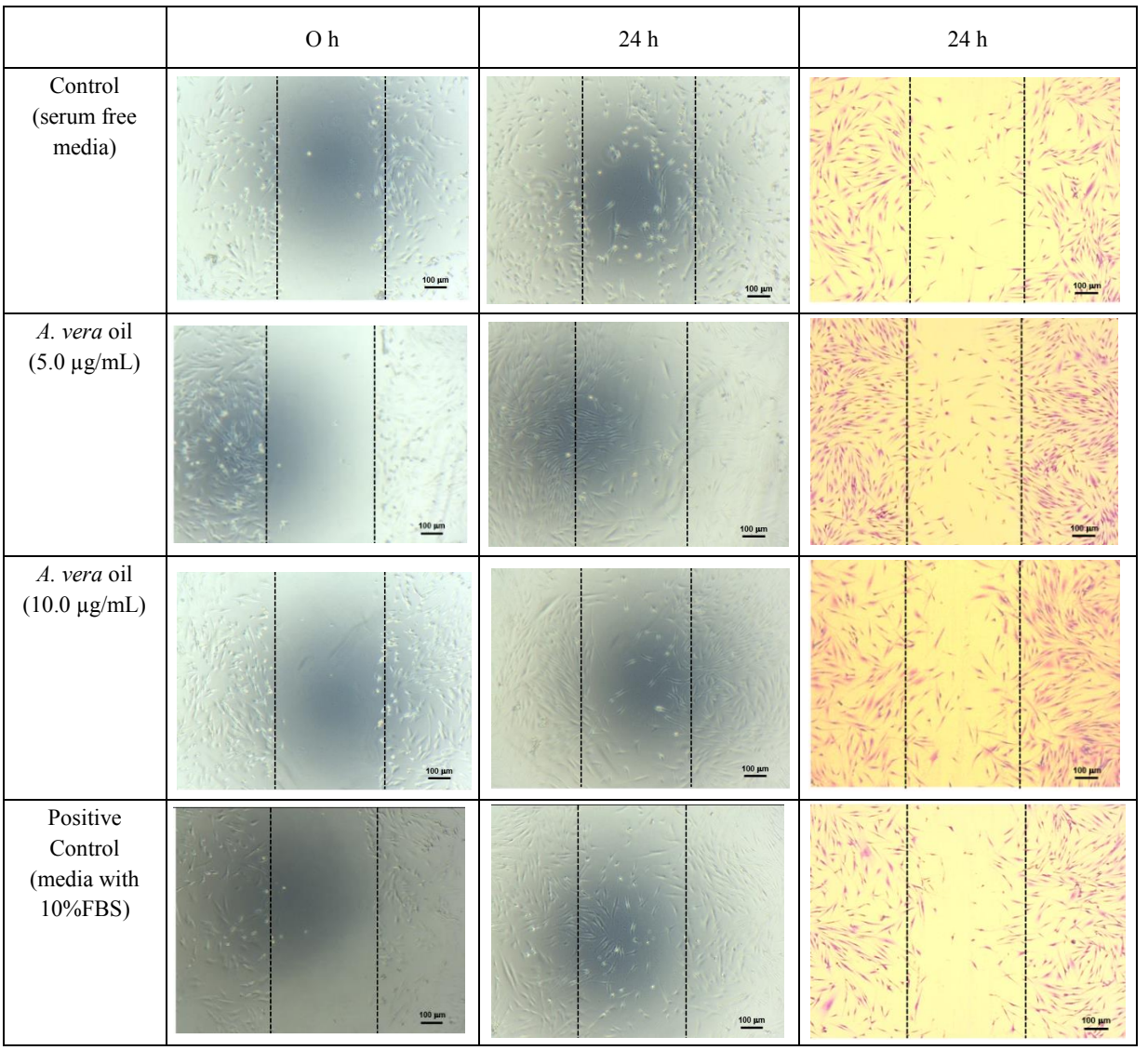

Fig. 7 Scratch wound healing assays were conducted to examine NHDF migration with or without *A. vera* oil at concentrations of 5 µg/mL and 10 µg/mL over a 24-h period. Representative images of the scratch wound are shown in phase contrast and brightfield modes. Scale bar: 100 µm

ingredient for cosmetic skincare applications. Moreover, treatment with *A. vera* oil enhanced NHDF cell migration and delayed the onset of phenotypic aging. The collective evidence indicates that *A. vera* oil possesses antioxidant and anti-inflammatory properties, underscoring its potential as a valuable component in skin cosmetic formulations. Future research will focus on evaluating the skin penetration and irritation potential of formulations containing *A. vera* oil, alongside clinical trials assessing its efficacy in skincare products.

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