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Original Article

Exploring the Potential of *Aloe vera* Hydro-Alcoholic Leaf Extract for Topical Diabetic Wound Healing Treatment

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Abstract

Background: Diabetic wounds present substantial challenges within the diabetic patient population, prompting diverse therapeutic strategies including the exploration of natural remedies. **Methods:** This investigation aims to comprehensively evaluate the efficacy of topical application of *Aloe vera* hydro-alcoholic leaf extract (AVHE) in addressing the various contributing elements to delayed wound healing in the diabetes through cell based assays and animal model. **Results:** The AVHE exhibited remarkable α-amylase inhibitory activity, indicated by an IC50 value of 0.051 mg/mL, comparable to the standard Acarbose (IC50 = 0.041 mg/mL). Fluorescent microscopy analysis demonstrated significant glucose uptake by McCoy fibroblast cells treated with AVHE, evidenced by 187.74% uptake of 2-NBDG at concentration of 0.1 mg/mL. Furthermore, the AVHE exhibited notable anti-bacterial efficacy against a spectrum of tested pathogens, with pronounced effectiveness against *Bacillus subtilis* and *Staphylococcus aureus*. The AVHE displayed enhanced wound closure, achieving a remarkable 57.03 % closure rate at concentration of 0.1 mg/mL compared to the untreated cells 10.01% in an *in vitro* scratch assay conducted in McCoy cells. *In vivo* assessments included evaluating the potential of AVHE to induce caudal fin regeneration in Zebra fish, revealing a significant 60% development within 7 days post-amputation. **Conclusion:** Collectively, these findings highlight the therapeutic potential of topical application of AVHE for the advancement of treatments targeting diabetic foot ulcers.

Keywords: *Aloe vera; Anti-Diabetic; Diabetic Wounds; in vivo*

Introduction

Diabetes mellitus (DM) poses a significant and widespread health challenge globally. Mismanagement of diabetes can result in a range of complications. Among these complications, diabetic foot ulcers (DFUs) are particularly concerning as they are linked with an elevated risk of mortality in individuals with diabetes (Tomic, Shaw & Magliano, 2022). DFUs, a leading cause of amputations, impact approximately 15% of individuals living with diabetes. These ulcers arise due to peripheral nephropathy and ischemia, which are consequences of peripheral vascular conditions (Yousif, 2024). Effective wound healing depends on the vital interplay between keratinocytes and fibroblasts, along with numerous biologically active molecules (Okur *et al.*, 2021). Multiple mechanisms come into play during the proliferative stage of the healing process in individuals with DFUs, resulting in reduced peripheral blood flow and impaired local angiogenesis. These factors include diminished cell and growth factor responses, all of which contribute to healing delays. Moreover, other elements such as delayed inflammatory response, microbial infection, and oxidative stress are also linked to the prolonged healing process in these cases (Mohsin *et al.*, 2024). The treatment of wounds in diabetic patients often involves the utilization of synthetic drugs, yet their application is constrained by significant limitations. Medicinal plants hold great promise as valuable resources in the search for novel agents to treat various

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conditions (Isopencu *et al.*, 2023). *Aloe vera* is an important facet of herbal medicine resources for treating primary healthcare. *A. vera*, a member of the Liliaceae family and the oldest and most widely used medicinal herb, is treasured in the cosmetics, pharmaceutical, and food industries. Its medicinal uses, which include the management of arthrosis, acne, and ulcers, are expanding quickly. *A. vera* can be utilized against oxidation stress caused by free radicals, inflammation, microbial infection, diabetes, and cancer, according to several reports. *A. vera* also exhibits nutritive, healing, moisturising, lubricating, and regenerating qualities (Chelu *et al.*, 2023a). *A. vera* gel contains polysaccharides, glycoproteins, and acemannan, all of which exhibit antibacterial and immunomodulatory potential, along with the ability to accelerate wound closure and promote tissue repair (Patitucci *et al.*, 2025).

This study aims to explore the effects of topical application of *A. vera* hydro-alcoholic extract (AVHE) on the healing of diabetic wounds, with a specific focus on multiple associated factors. By employing both *in vitro* and *in vivo* approaches, the current study offers scientific validation for the safe use of AVHE in the pharmaceutical sector as a potential intervention to enhance the healing process of DFUs.

Materials and Methods

Materials

The hydro-ethanolic extract of leaves of *Aloe barbadensis Miller (A. vera)* was procured from Konark Herbals and Health Care, Mumbai, Maharashtra, India. McCoy fibroblast cells were procured from National Centre for Cell Science, Pune, India. Mueller Hinton agar, Mueller-Hinton broth, and 96-well bottom plates, Dulbecco's modified eagle medium (DMEM), Fetal bovine serum (FBS), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and penstrep were acquired from Himedia (India). 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose (2-NBDG) Molecular Probes were purchased from Invitrogen. Unless specifically stated otherwise, all chemicals were sourced from Sigma Aldrich.

Estimation of Proximate composition

The methodology for assessing moisture content, total ash, acid-insoluble ash, water-soluble extractives, and ethanol-soluble extractives in AVHE adhered to the recommended guidelines of WHO (1998).

Qualitative screening of phyto-chemicals

To detect the presence of various phytochemicals including carbohydrates, proteins, alkaloids, tannins, flavanoids, phenols, and sterols in AVHE, several assays were conducted, as presented in Table 1 (Bhardwaj & Dubey, 2019).

Table 1: Test for qualitative analysis of AVHE

Test	Methodology	Observation
Carbohydrates	AVHE + alpha-napthol (3-5 drops) + H_2SO_4	Violet ring at the intersection of layers
Proteins	AVHE + Biuret reagent	Color change from blue to deep violet or pink
Alkaloids	AVHE + 2 mL HCl (2%) + Wagner's reagent	Reddish brown precipitate
Tannins	AVHE + 1 mL $FeCl_3$ (3%)	Appearance of brownish green color
Flavonoids	AVHE + NaOH (5%) + 2 mL HCl (10%)	Color change from yellow to colorless
Phenols	AVHE + $FeCl_3$ (5%)	Appearance of dark green or blue color
Sterols	AVHE + Conc. H_2SO_4 (2 mL)	Red precipitate
Saponins	AVHE + dist. H_2O (Shaking for 15 min)	Formation of 1 cm long foam

Quantitative analysis of the phyto-chemicals

Carbohydrate estimation: The amount of carbohydrate in AVHE was calculated using the Anthrone method. AVHE (1 mL) and anthrone reagent (4 mL) were mixed together and boiled for 8 min in a water bath. At 630 nm, the optical density was recorded against blank (Hedge, Hofreiter & Whistler, 1962).

Protein estimation: Assessment of the protein content was done using the Lowry method. Briefly, freshly made alkaline copper reagent (4mL) was mixed with AVHE (1 mL) and allowed to stand at room

temperature. Following that, after 10 min, 0.5 mL of Folin-Ciocalteau (FC) reagent (1N) was added to the mixture and dark incubation for another 30 min was given. Afterwards, at 660 nm, absorbance was measured (Lowry *et al.*, 1951).

Total phenolic content (TPC): By using the FC test, the TPC was measured. Gallic acid was used at various concentrations to establish a standard curve (Singleton, Orthofer & Lamuela-Raventós, 1999).

Total flavonoid content (TFC): Employing aluminium chloride calorimetric approach, TFC was measured. Different quercitin concentrations were used to generate the calibration curve. Absorbance at 510 nm was measured (Woisky & Salatino, 1998).

Study of Anti-oxidant Activity

2–2'-azino-di-(3-ethylbenzthiazoline sulfonate) ABTS: ABTS radical scavenging test was utilized to evaluate the anti-oxidant properties of the AVHE. The ABTS radical cation stock was prepared by mixing ABTS solution (7 mM) and potassium persulfate solution (2.45 mM) together in a 1:1 ratio. ABTS working solution with absorbance of 0.70 was prepared by diluting with methanol. For conducting the assay, AVHE was added to 2.9 mL of ABTS working solution and incubated for 6 min. Then the OD of reaction mixture was recorded at 734 nm. To plot the standard curve different concentrations of ascorbic acid were used (Re *et al.*, 1999).

ABTS inhibition percentage was calculated as follow:

$$\text{Percent inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2,2-diphenyl-1-picrylhydrazyl (DPPH): In order to conduct this test, range of concentrations of AVHE were added to 0.1 mM DPPH solution, and dark incubation at 37°C was given. Using a spectrophotometer, after 30 min the OD of reaction mixture was read at 517 nm. Butylated hydroxytoluene (BHT) was used in various concentrations to construct the standard plot (Blois, 1958).

Percentage DPPH inhibition was calculated as follow:

$$\text{Percent inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Study of Anti-diabetic Activity

Dinitrosalicylic acid (DNSA) assay for α-amylase inhibition: Using the DNSA technique, the α-amylase inhibitory test was conducted (Sumner & Graham, 1921). As a positive control, Acarbose was used. Varying concentrations of AVHE were dissolved in phosphate buffer saline (PBS). α-Amylase (2 units/mL) was mixed with AVHE, and incubation at 30°C was given for 10 min. Afterwards, the starch solution was added to reaction mixture, further incubated for 10 min at 30°C, and boiled for 10 min after the addition of DNSA reagent. The absorbance at 540 nm was recorded.

The α-amylase inhibitory activity was expressed as percent inhibition and was calculated as follow:

$$\text{Percent Inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2-NBDG uptake in McCoy cells: McCoy fibroblast cells were cultured in 96-well flat bottom plates in DMEM, 10% FBS, and 1% penstrep in humidified incubator at 37℃ and 5% CO_2. Following 15 min pretreatment with AVHE (0.05 and 0.1 mg/mL) and Calendula cream (0.1 mg/mL), a fresh medium containing 100 µM of fluorescent glucose derivative 2-NBDG was supplemented. The cells were then incubated for 1 h at 37℃. Afterward, the cells were washed with cold PBS, and the fluorescence within the cells was observed using a fluorescent microscope (Nikon Eclipse Ti-U) with excitation and emission at 490 nm and 525 nm, respectively. The mean fluorescence intensity of different groups was analyzed using ImageJ software (Rasband, 2018).

Human red blood cell (HRBC) membrane stabilization assay

The stabilization of the membrane of HRBC has been employed as a technique to examine the *in vitro* anti-inflammatory efficacy. Whole blood was collected from a healthy donor and centrifuged at 2500

rpm for 5 min in heparinized centrifuge tubes, followed by three times washing with normal saline solution. Thereafter, 10% v/v erythrocyte suspension was reconstituted with normal saline. The 3 mL reaction mixture comprised of 50 µL of AVHE, 50 µL of blood cell suspension and 2.9 mL of PBS (7 pH). Incubation of 20 min at 54°C was given to the reaction mixture, afterwards, centrifuged for 3 min at 2500 rpm, and the OD of the supernatant was read at 540 nm. Aspirin was used as a positive control.

The level of protection was calculated as follow:

$$\text{Percent protection} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Study of Anti-bacterial Activity

The AVHE was assessed for its activity against bacteria commonly implicated in causing infections at wound sites (*Staphylococcus aureus* MTCC3160, *Bacillus subtilis* MTCC121, *Escherichia coli* ATCC25922 and *Salmonella typhi* MTCC98) in accordance with the guidelines of CLSI, 2012.

By using agar-well diffusion method, the anti-bacterial potential of AVHE was determined. The antibiotic Ampicillin (0.02 mg/mL) was employed as positive control. Each bacterial strain suspension was spreaded over the Mueller Hinton agar and wells with diameter of 5 mm were punctured and loaded with different concentrations of AVHE. These plates were then incubated overnight at 37℃ and the zones of inhibition were measured.

Furthermore, by using micro-broth dilution method, the minimum inhibitory concentration (MIC) of AVHE was determined. In 96-well plate containing sterile Mueller-Hinton broth, AVHE was serially diluted, followed by the addition of bacterial suspension. After that, incubation at 37°C for 24 h was given to the 96-well plates. Thereafter, resazurin dye (0.04% w/v) was added to each well, plates were then incubated until the color of the media changed (blue to pink), signifying the growth of bacterial cells.

Study of cell proliferation and migration

The cell-proliferation was assessed by MTT assay. Briefly, McCoy cells were seeded at 10^4 cells/well in 96-well plate in DMEM containing 0.12% FBS. Cells were exposed to various concentrations of AVHE. After the incubation of 24 h, MTT (5 mg/mL) was added to each well and further incubation for 4 h at 37℃ was given. The media from the wells was then aspirated and dimethyl sulfoxide (DMSO) was added, and the absorbance was read at 580 nm.

Afterwards, the wound scratch assay was utilized to evaluate the effect of AVHE on migration of McCoy fibroblasts. In 24-well tissue culture dishes, the cells were seeded at a density of 3×10^4 cells/mL and grown in media containing 10% FBS until they nearly formed confluent cell monolayer. A sterile 100 µL pipette tip was then used to create a linear wound in the monolayer. To eliminate any cellular debris, the wells were washed with PBS. Different treatments including a control group with DMEM only, Calendula cream (0.1 mg/mL) as the positive control, and AVHE at concentrations of 0.05 and 0.1 mg/mL, were given to the cells. These treated scratches were then incubated at 37°C with 5% CO2 overnight. Images of the scratched areas were captured after incubation to determine the relative migration of cells. The data collected from the images were analyzed using ImageJ software (Rasband, 2018).

Evaluation of wound healing potential in Zebra fish

Adult Zebra fish could be used as a model system to assess the wound healing efficacy of bio-active compounds and drugs (Richardson *et al.*, 2013). Prior to commencing the experiment, a two-week acclimatization period was provided for the Zebra fish to adapt to the laboratory environment. The zebra fish population was segregated into four distinct groups: control, standard, and two experimental, with each group consisting of five individual fish. These fish were given a 1% glucose solution environment to induce a hyperglycemic environment. To initiate the experimental procedure, each fish was anesthetized using clove oil (0.02%) after which a sterile surgical blade was employed to make a transection of the caudal fin, approximately 5mm from the posterior end. Subsequent to the fin

transection, the fish received treatment involving the application of the AVHE (2 mg and 4 mg) and Calendula cream (4 mg) from day 0 to day 7 using gloved hands. The ability of fish to regenerate the fin tissue over a period of seven days following the fin transection was utilized to assess the effect of AVHE on regenerative potential of the fins by measuring the fin growth.

Percentage of fin regeneration was calculated as:

$$\% \text{ Fin regeneration} = \frac{Fin\ growth\ on\ 7th\ day}{\text{Initial wound size}} \times 100$$

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics 20. The data were presented as the mean ± standard deviation (S.D.) and analyzed using a t-test. Significant differences between groups were determined at $P < 0.005$ levels.

Data Availability

All data generated or analyzed during this study are included in this published article.

Results

Proximate analysis

The proximate quantification of Moisture, Ash, Water and Ethanol soluble extractive were done to figure out medicinal importance of AVHE. AVHE showed moisture content of 2.9 ± 0.2%. The estimated total ash content of AVHE was found to be 3.5%, while the estimated acid-insoluble ash in the respective plant extract was determined to be 0.9%. The alcohol soluble and water soluble extractives in AVHE were revealed to be 87.1±0.2% and 85.8±0.2%, respectively.

Phytochemical screening

Tests for proteins, alkaloids, tannins, flavonoids, phenols, and sterols were performed as part of the phytochemical analysis. The AVHE showed the presence of all the studied metabolites including carbohydrates, proteins, alkaloids, tannins, flavonoids, phenols and sterols.

Furthermore, AVHE was analyzed to determine its total carbohydrate, protein, phenol, and flavonoid composition. It showed a total carbohydrate content of 11.51% and a protein content of 5.94%. A considerable level of phenols and flavonoids was found in AVHE. The TPC and TFC were evaluated and expressed as mg gallic acid equivalents/g of dry weight (mg GAE/g DW) and mg quercetin equivalents/g of dry weight (mg QE/g DW), respectively. AVHE was found to contain 118.01±0.23 mg GAE/g DW of phenols and 98.98±0.17 mg QE/g DW of flavonoids.

Anti-oxidant Activity of AVHE

In the current investigation, AVHE was examined to determine its anti-oxidant activity using ABTS and DPPH scavenging assays. In the process of conducting the ABTS assay, AVHE exhibited its most notable inhibitory effect at a concentration of 0.1 mg/mL, with an inhibition activity of 89±0.25% (Table 2). The IC50 value for the ABTS radical scavenging activity of AVHE was determined to be 0.046 mg/mL, and the standard (ascorbic acid) to be 0.026 mg/mL.

Table 2: Anti-oxidant activity of AVHE

Concentration (mg/mL)	% Anti-oxidant activity	
	ABTS	DPPH
0.025	36.43±0.43	31.5±0.37
0.05	56.33±0.16	45.42±0
0.075	72±0.14	68.74±0.21
0.1	89±0.25	83.64±0.21
IC50	0.046 mg/mL	0.052 mg/mL

As shown in Table 2, DPPH radical scavenging activity of AVHE demonstrated a significant increase with rising extract concentration. The highest DPPH radical scavenging activity (83.64±0.21 %) was

observed at concentration of 0.1 mg/mL. The anti-oxidant potency of AVHE displayed remarkable parallels to BHT, a standard reference. The IC50 values for DPPH inhibition were 0.044 mg/mL for the standard and 0.052 mg/mL for AVHE.

Hypoglycemic effect of AVHE

α-amylase inhibition: The α-amylase inhibition activity of the AVHE was investigated across different concentrations, as depicted in Figure 1. The findings indicate an increase in the percentage of inhibition that aligns with the concentration in a dose dependent manner. In relation to the employed standard Acarbose (85.43%), the AVHE exhibited a closely comparable inhibition of α-amylase activity (78.52%) at concentration of 0.1 mg/mL. The IC50 values obtained for the AVHE and Acarbose were 0.051 mg/mL and 0.041 mg/mL, respectively.

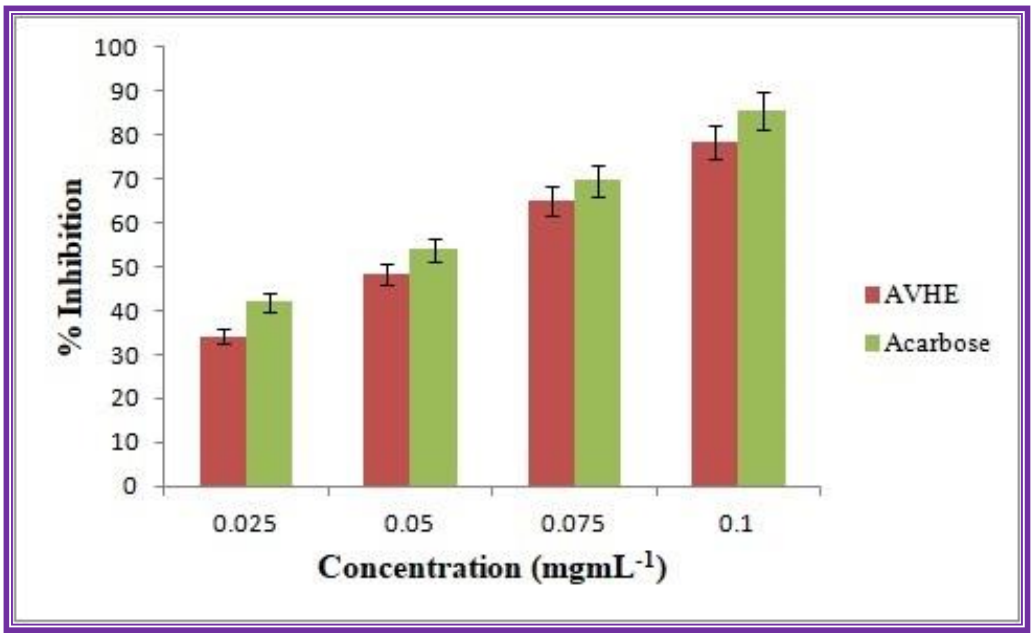
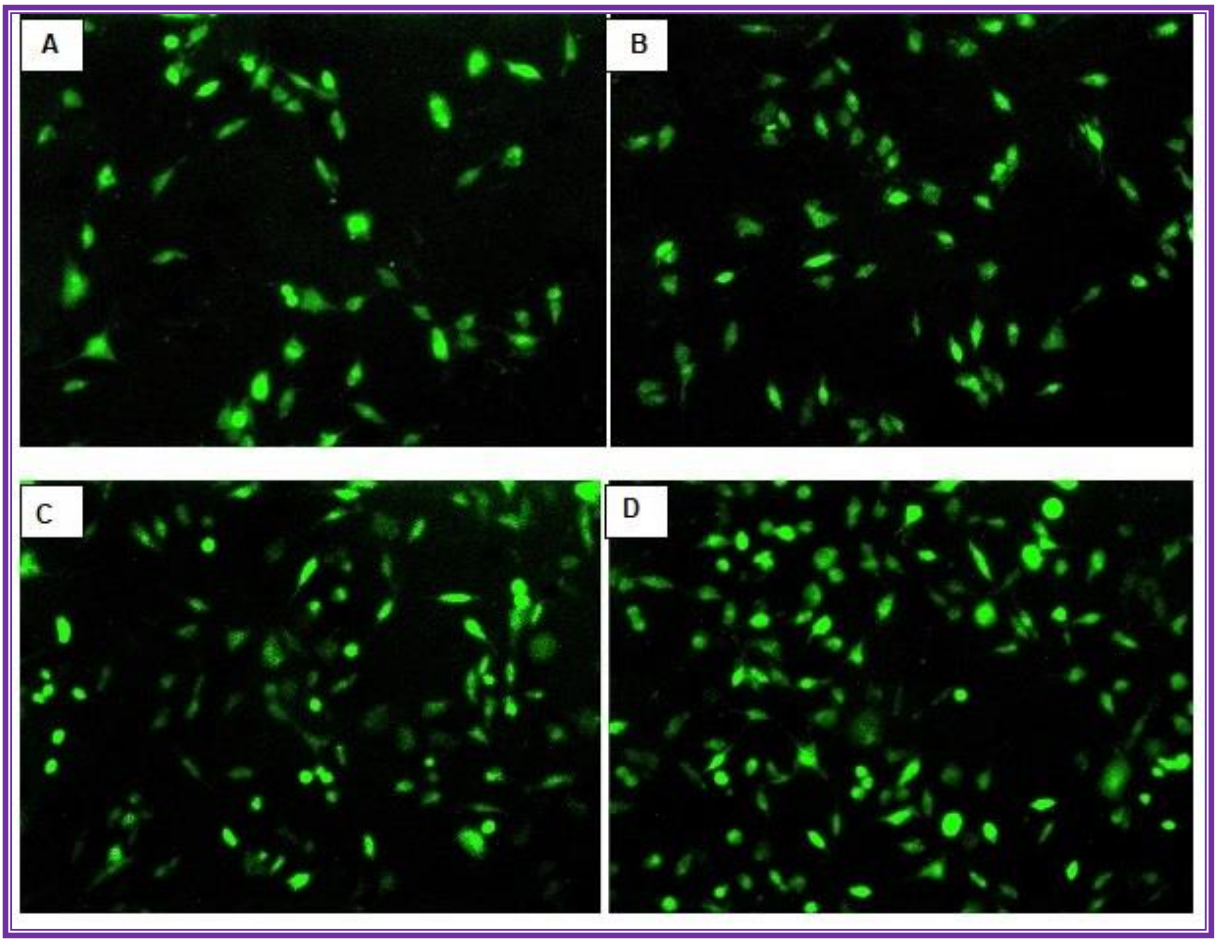


Figure 1: Anti-diabetic activity of AVHE: Graphical representation of inhibition of α-amylase activity of Acarbose and AVHE at various concentrations

2-NBDG uptake: The impact of AVHE on cellular glucose uptake in McCoy cells was examined in this study. Within the scope of the current investigation, McCoy fibroblast cells were subjected to treatment with AVHE at concentration of 0.05 and 0.1 mg/mL, over duration of 24 h. Notably, AVHE demonstrated the most efficient glucose uptake, as indicated by the vivid fluorescence displayed in Figure 2a-e. This level of uptake was comparable to that of the standard Calendula cream (0.1 mg/mL). The fluorescent intensity present in the images was subsequently evaluated through the utilization of Image J software. Cells subjected to AVHE treatment indicated a notably high relative fluorescence of 150.29 and 187.74% at concentrations of 0.05 and 0.1 mg/mL, respectively, as compared with the positive control, which displayed a relative fluorescence of 143.73%. Cellular glucose uptake (Figure 2) was enhanced by non-cytotoxic concentration of AVHE.



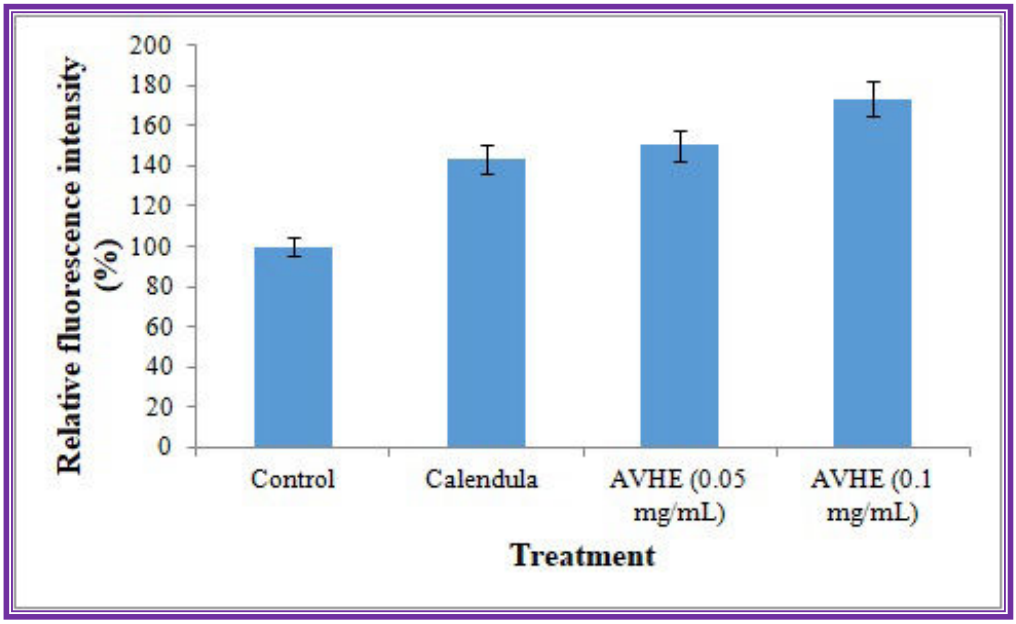


Figure 2: 2-NBDG uptake assay in McCoy fibroblast cells by fluorescent microscopy: (A) untreated cells, (B) treated with Calendula cream, (C) treated with 0.05 mg/mL of AVHE, (D) treated with 0.1 mg/mL of AVHE, and (E) fluorescence intensity graph

Anti-inflammatory effect of AVHE

Stabilization of HRBC membranes was investigated to further determine the anti-inflammatory potential of AVHE. AVHE inhibited heat-induced hemolysis of RBCs, with maximum anti-inflammatory effects shown by AVHE (69.72±0.45%) at a concentration of 0.2 mg/mL as shown in the Figure 3. The IC50 values for AVHE and Aspirin were 0.130 mg/mL and 0.083 mg/mL, respectively.

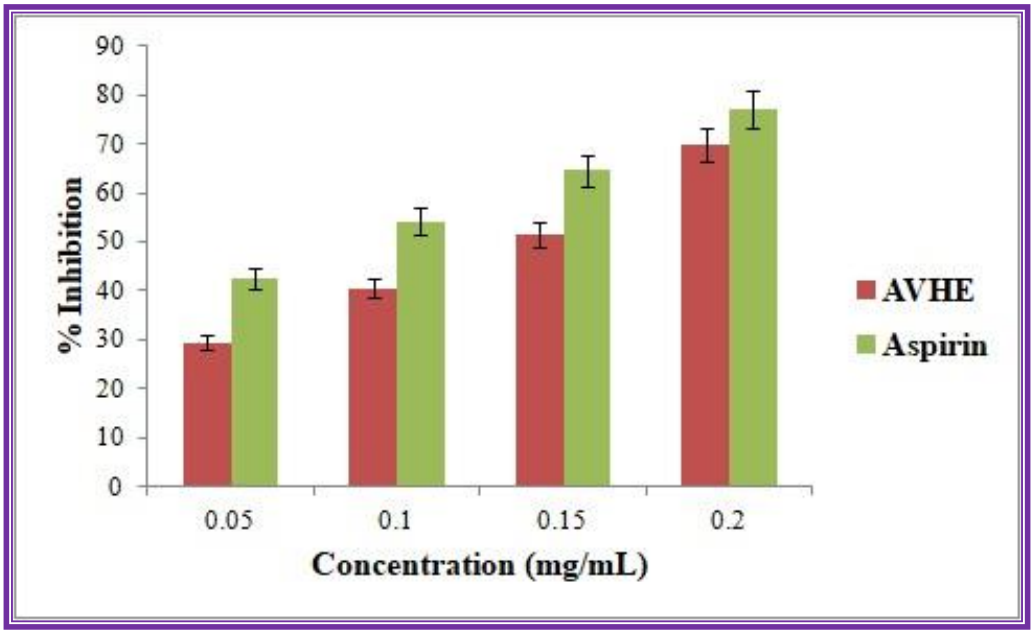


Figure 3: Anti-inflammatory activity of AVHE: HRBC membrane protection/ stabilization potential of AVHE and Aspirin

Anti-bacterial Activity of AVHE

The agar-well diffusion method was employed to assess the anti-microbial efficacy of AVHE against bacterial strains. This involved measuring the inhibition zone diameter. The mean inhibition zone of AVHE and ampicillin is shown in Table 3, Figure 4a-d. The results revealed that the AVHE exhibited considerable anti-bacterial activity against all the selected strains of microorganisms tested at a concentration of 0.9 mg/mL.

The MIC of AVHE was determined using broth micro-dilution method. The MIC for AVHE ranged between 0.781 and 3.125 mg/mL and that of Ampicillin ranged between 0.0156 and 0.0312 mg/mL (Table 3).

Table 3: *Anti-bacterial activity of AVHE against pathogenic bacteria*

Bacterial strain	**Zone of inhibition (mm)**		**MIC (mg/mL)**	
	AVHE (0.9 mg/mL)	**Ampicillin (0.2 mg/mL)**	**AVHE**	**Ampicillin**
***S. aureus* (MTCC3160)**	14±0.14	18.5±0.21	0.781	0.0156
***B. subtilis* (MTCC121)**	14.65.1±0.14	18.25±0.21	3.125	0.0312
***E. coli* (ATCC25922)**	14.9±0.14	18.0±0	3.125	0.0312
***S. typhi* (MTCC98)**	13.35±0.35	17.75±0.14	1.562	0.019

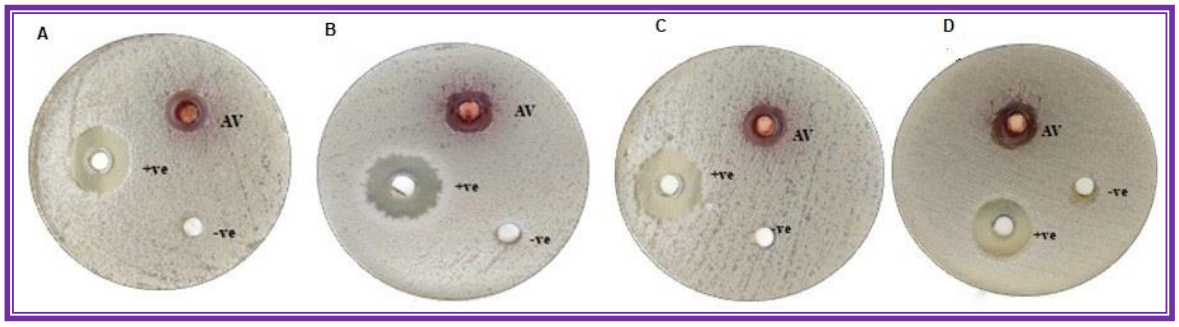


Figure 4: Anti-bacterial activity of AVHE: Zone of inhibition (mm) obtained by agar well diffusion method for AVHE and Ampicillin against (A) *S. aureus*, (B) *B. subtilis*, (C) *E. coli*, and (D) *S. typhi.*

In vitro proliferative and migrative effect of AVHE on fibroblast cells

From our results (Figure 5), after treatment with AVHE, significant cell proliferation effects were observed in McCoy fibroblasts. By comparing the results from the AVHE treated cells with the standard (Calendula cream) and untreated cells (control), the proliferation potential was estimated. The cells subjected to an AVHE concentration of 0.1 mg/mL exhibited the most substantial degree of cell proliferation, showcasing a notable increase of 145.76%. This corresponds to a threefold rise in the proliferation rate compared to the untreated cells. On the other hand, cells treated with Calendula cream exhibited twofold increase in proliferation rate.

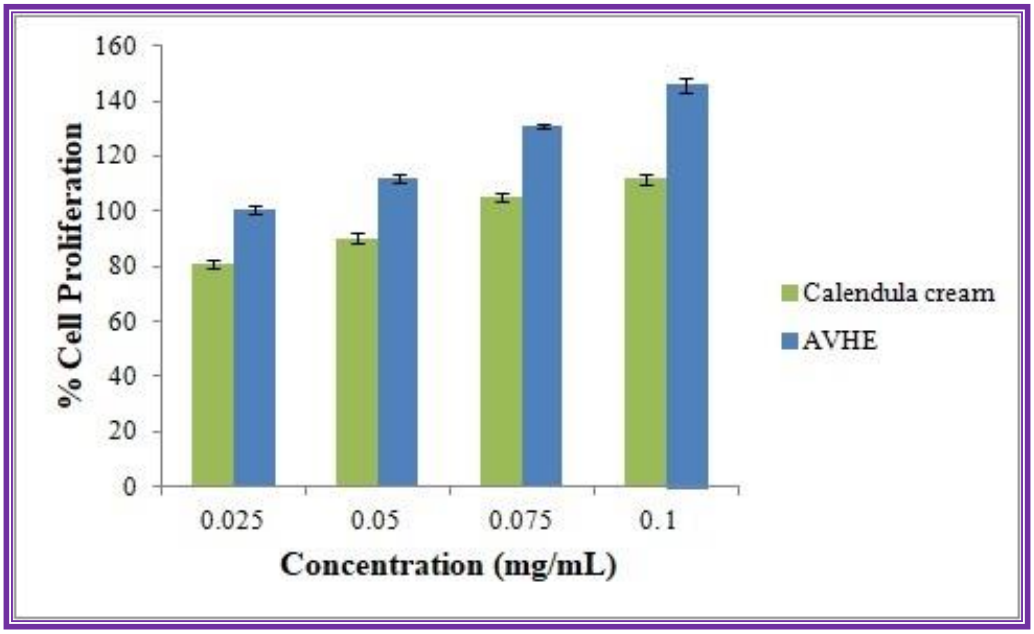


Figure 5: Effect on proliferation of McCoy fibroblast: graphical representation of the evaluation of Calendula cream and AVHE effects

In Figure 6a-f, the depicted data showcases the percentage and microscopic images of wound closure outcomes observed in cells when subjected to AVHE (0.05 and 0.1 mg/mL), standard (Calendula cream; 0.1 mg/mL), and an untreated control. Our findings indicated that, within a 24-h period, fibroblasts exhibited proliferation and migration towards the scratched region in the AVHE treated group (Figure 6a-f), which led to a substantial reduction in the wound area. In the case of cells treated with AVHE, a 42.82 and 57.03% relative wound closure area was observed at concentrations of 0.05 and 0.1 mg/mL, respectively, while for Calendula cream treatment, it amounted to 39.81%.

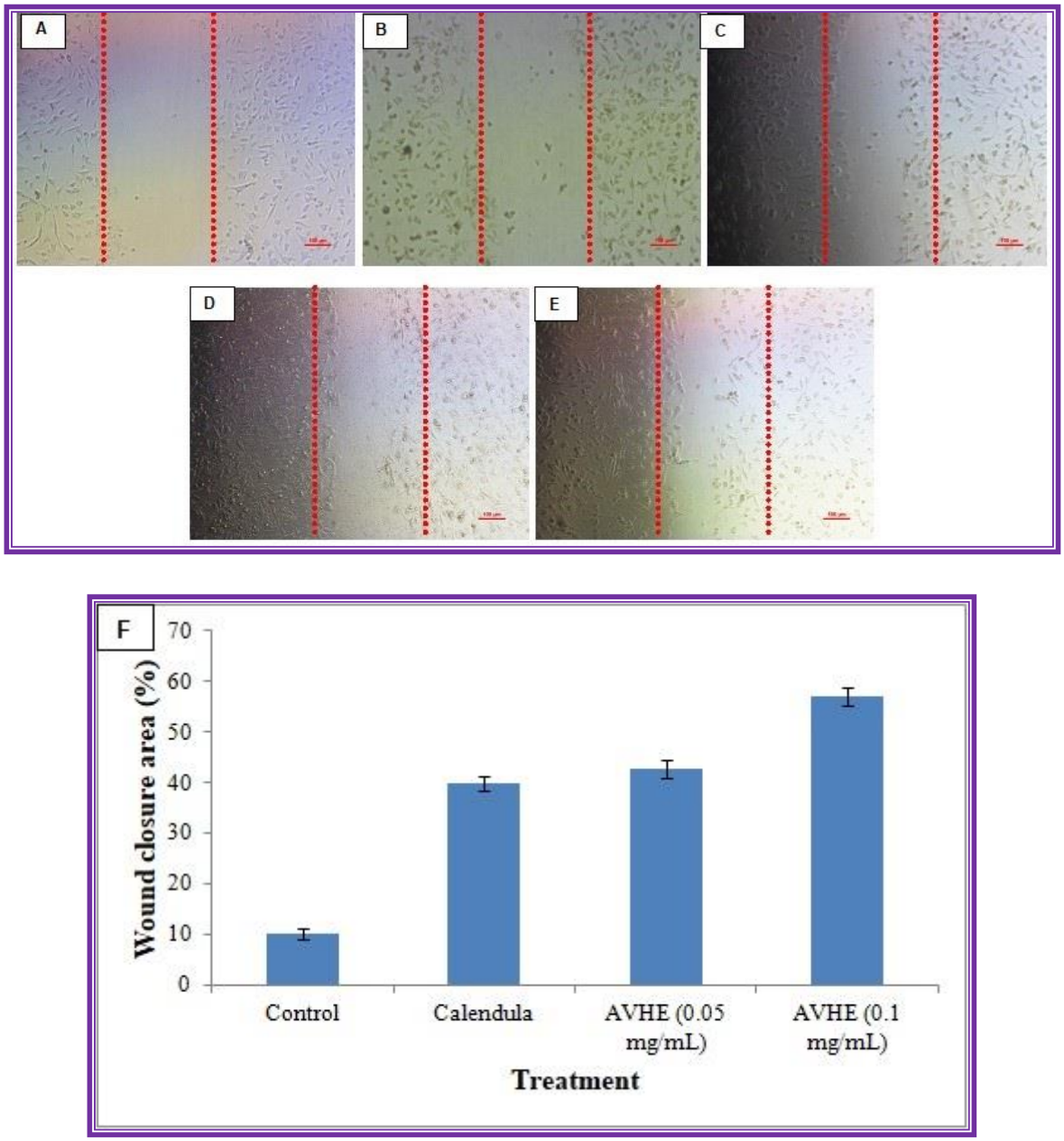


Figure 6: Effect on migration of McCoy fibroblast: Micrograph from scratch assay: (A) fibroblasts immediately after scratch creation, (B) untreated fibroblasts 24 h post scratch, (C) Calendula cream-treated fibroblasts 24 h post scratch, (D) 0.05 mg/mL of AVHE treated fibroblasts 24 h post scratch, (E) 0.1 mg/mL of AVHE treated fibroblasts 24 h post scratch, and (F) quantification of migrated fibroblast toward the scratch area.

Effects of AVHE on fin regeneration of Zebra fish

The growth of the fins in the AVHE-treated group and the control group were compared seven days after transection. The group that received AVHE treatment recovered better with fin regeneration rates of 52.67 and 60% at doses of 2 mg and 4 mg, respectively, compared to the group that received the standard treatment of Calendula cream (45%), as demonstrated in Figure 7a–e.

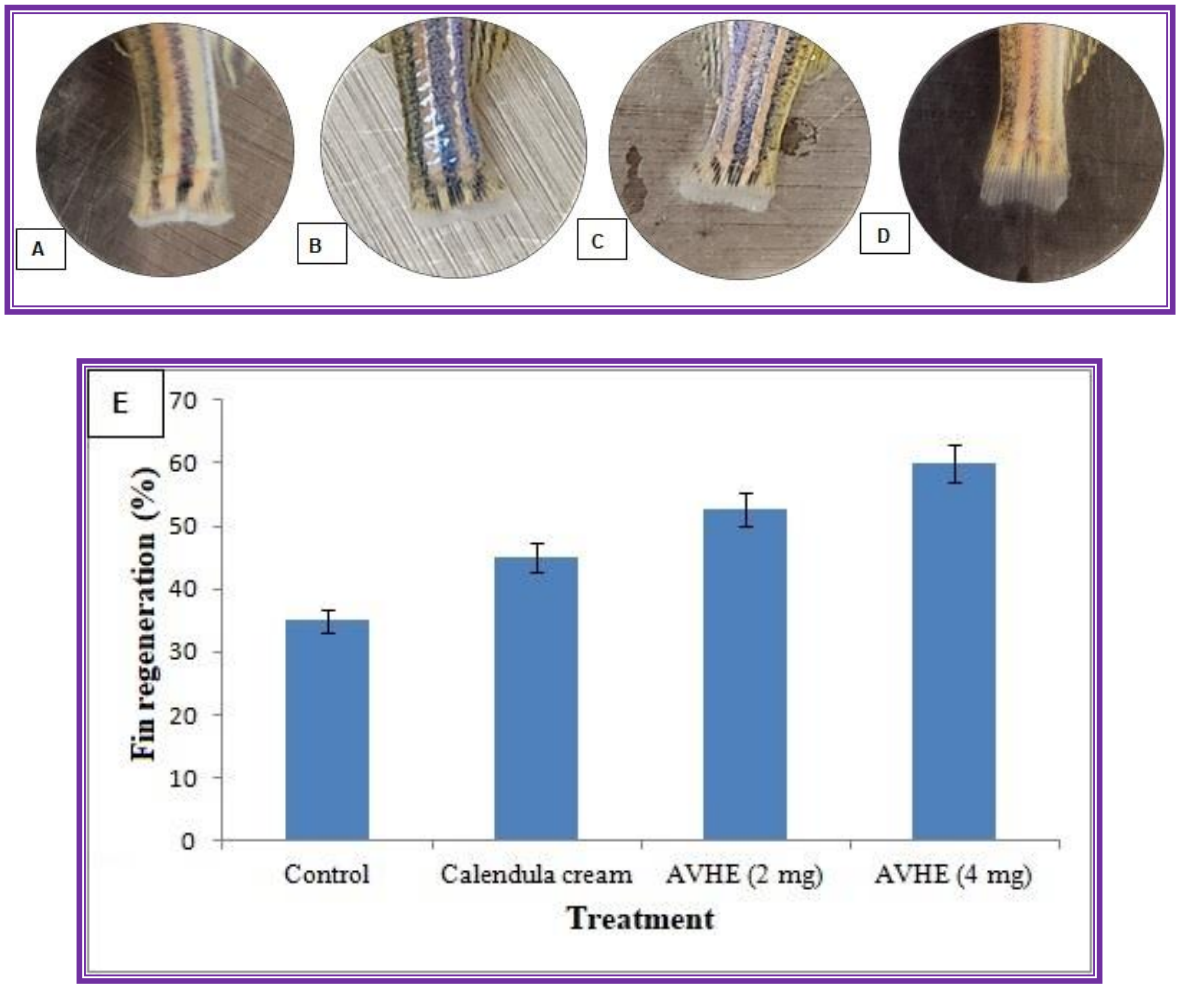


Figure 7: Comparative evaluation of fin regeneration on the 7th day post transection: (A) untreated Zebra fish, (B) Zebra fish treated with Calendula cream, (C) Zebra fish treated with 0.05 mg/mL of AVHE, (D) Zebra fish treated with 0.1 mg/mL of AVHE, and (E) graphical representation illustrating the effects of various treatments on fin regeneration of Zebra fish.

Discussion

Plant-derived bioactives have demonstrated promising wound healing potential by accelerating epithelization and minimizing scar formation. Although wound healing is a natural immunological response, its efficiency can be compromised by several factors such as high glucose levels, inflammation, oxidative stress, and microbial colonization. Conventional therapies frequently fail to sufficiently aid tissue repair, emphasizing the need for novel, multifaceted treatments (Kaczmarek-Szczepańska *et al.*, 2025). Thus, plant based compounds provide a non-toxic, safer, and biocompatible alternative that promotes faster wound contraction. The multifactorial nature of DFU's particularly is challenging to treat, as various intrinsic and extrinsic factors interplay to impede the healing process. Therefore, an attempt was made to evaluate the wound healing activity of AVHE.

The chemical analysis of AVHE revealed a low moisture level and high ash content. Low moisture levels are advantageous for wound dressings since excess moisture may delay healing, particularly in diabetic wounds that are more prone to infection. High ash content is a valuable parameter in proximate analysis used to assess the mineral composition of a sample. Ashing is the first step in preparing a biological sample for a specific elemental analysis. Due to the high concentrations of certain minerals in some samples, ash content analysis becomes an important aspect (El Hosry *et al.*, 2023). These findings reveal significant details regarding the chemical composition of AVHE, which, in turn may contribute to its potential therapeutic applications in the treatment of diabetic wounds. Numerous bioactive

phytocompounds, such as polysaccharides, carbohydrates, minerals, proteins, lipids, and phenolic compounds, are abundant in *A. vera* gel (Chelu *et al.*, 2024). Defence mechanisms against insects, pathogens, and other herbivores are directly impacted by these phyto-chemicals and other aromatic compounds. The diverse therapeutic properties of *A. vera*, including its wound healing, antifungal, anti-inflammatory, immunomodulatory, anticancer, and antibacterial activities, are attributed to the synergistic interactions among its numerous bioactive constituents (Girgin *et al.*, 2024).

In the current investigation, AVHE was discovered to contain 11.51% carbohydrates. These act as a primary energy source that supports cellular metabolism and tissue regeneration. Furthermore, they contribute to the structural and textural properties of wound dressings, thereby impacting on wound healing. Notably, the mannose-6-phosphate and acemannan polysaccharides in Aloe gel play a crucial role in stimulating fibroblast and macrophage proliferation, ultimately enhancing collagen biosynthesis (Liang *et al.*, 2021). The protein content in AVHE was found to be 5.94%, further supporting its role in collagen synthesis, regulation of immune response, and tissue repair, highlighting the potential applications of AVHE in wound care therapies.

Furthermore, the extract was rich in flavonoids and phenolic compounds. These secondary metabolites exhibit potential anti-bacterial, anti-inflammatory, collagen-stimulating, anti-oxidant, and angiogenic properties, enhancing wound repair. The presence of phenols and flavonoids highlights the importance of employing plant-derived compounds to support the complex and impaired process of wound healing. In Aloe and other plants, phenolic compounds are significant secondary metabolites. These compounds belong to the category of polyphenols and possess phenolic hydroxyl groups attached to ring structures that enable them to serve as anti-oxidants (Floris *et al.*, 2021).

One of the primary mechanisms by which hyperglycemia impairs wound healing is through the overproduction of reactive oxygen species (ROS) via the protein kinase C, hexosamine, and polyol pathways (Chen *et al.*, 2025). Although ROS are necessary for the early stages of wound healing, an imbalance in the generation of ROS has been demonstrated to be detrimental to the later stages. In the current study, the anti-oxidant activity of AVHE was evaluated against DPPH and ABTS free radicals (Table 2). These methods are widely used to assess anti-oxidant potency by determining IC50 value — the quantity of plant extract required to achieve a 50% reduction in the initial DPPH/ ABTS concentration. IC50 is inversely proportional to the anti-oxidant activity i.e., stronger anti-oxidant activity of the studied sample is indicated by a lower IC50 value. Saeed *et al.*, (2022), evaluated the DPPH radical scavenging activity of *A. vera* extracts, finding that the methanol extract prepared was more potent than the water extract. This anti-oxidant activity is attributed to its substantial polyphenolic content. Anti-oxidants play a crucial role in mitigating various illnesses associated with oxidative stress, including cancer, cardiovascular diseases, nerve degeneration, and diabetes (Akbari *et al.*, 2022). Thus, the anti-oxidant properties of AVHE potentially promote wound healing by reducing oxidative stress and creating an optimal environment for tissue regeneration.

Since hyperglycemia in diabetic individuals relates to impaired wound healing and the development of DFU, the present study also explored the anti-diabetic effect of AVHE. Given that carbohydrate digestion commences in the mouth and persists in the intestine, catalyzed by salivary and pancreatic α-amylase — inhibitors of this enzyme can aid in the management of hyperglycemia. Acarbose, a standard medication, showcases significant α-amylase inhibition activity, resulting in the delay of starch hydrolysis into oligosaccharides and disaccharide (Hajfathalian, Ghelichi & Jacobsen, 2025). The glucose lowering effect could be further explained on the basis of anti-oxidant properties of AVHE extracts which attenuates the oxidative damage. Our findings have shown that inhibition of α-amylase observed with AVHE may be attributed to the presence of phenolic and flavonoid compounds, known for their enzyme inhibitory and glucose lowering effect (Babu *et al.*, 2019).

To further validate the anti-diabetic potential, glucose uptake in McCoy cells was assessed using 2-NBDG, a fluorescently labeled analog of deoxy-glucose, which has found utility in tracking glucose assimilation within viable cells. Impaired insulin signaling, referred to as insulin resistance, stands as one of the predictors for the development of type 2 diabetes. Once hyperglycemia sets in, insulin

resistance becomes a focal point for therapeutic intervention (Berbudi, Khairani & Tjahjadi 2025). Our findings demonstrated that AVHE significantly enhances glucose uptake in McCoy cells, implying strong therapeutic and potential anti-diabetic effects. The observed increase in glucose uptake may be attributed to the presence of bioactive phytocompounds in AVHE.

Another factor that prevents diabetic patient's wounds from healing is chronic inflammation, which is a natural physiological response that occurs after injury to tissue or wound formation. Persistent inflammation interrupts the natural healing process, resulting in severe tissue damage. In this context, AVHE's anti-inflammatory potential was assessed using erythrocyte membrane stabilization, a well acknowledged model due to the similarity between RBC membranes and lysosomal membranes. Damage to these membranes, which is frequently caused by ROS mediated lipid peroxidation, raises the risk of cell damage. Phytochemicals with membrane-stabilizing properties can help prevent this damage by reducing phospholipase activity and thus inflammatory mediator release (Sena *et al.*, 2025). In line with these findings, the current investigation found that AVHE has a significant anti-inflammatory impact, with results demonstrating up to 69.7% suppression of inflammatory activity. The anti-inflammatory mechanism is believed to involve inhibiting the cyclooxygenase (COX) pathway, which reduces the production of prostaglandin E2 from arachidonic acid (Mahesh, Anil Kumar & Reddanna, 2021). Effective inflammation management is critical in wound healing because it prevents further tissue damage and stimulates progression to the proliferative and remodelling phases. The anti-inflammatory activity of AVHE not only supports its traditional use in wound care, but also emphasizes its importance in modern therapeutic approaches for addressing complex wounds such as DFUs.

Another cause of delayed healing in diabetic wounds is bacterial colonization, which acts as a significant extrinsic factor disrupting the natural healing cascade. Persistent microbial presence at the wound site can worsen inflammation, prevent granulation tissue formation, and raise the risk of infection-related consequences. While topical antibacterial medicines are widely used to treat infections, several have been shown to have a deleterious impact on wound healing by being cytotoxic to regenerating tissues (Muller *et al.*, 2003). *A. vera*, on the other hand, has the unique ability to promote wound healing while simultaneously serving as an anti-microbial therapeutic. The distinctive characteristic of the *A. vera* plant lies in its noted anti- bacterial activity against diverse pathogens (Chelu *et al.*, 2023b). In the current study, AVHE was evaluated against pathogenic bacteria and found to efficiently inhibit bacterial growth (Table 3, Figure 4). This indicates strong antibacterial activity, potentially due to the presence of bioactive compounds such as anthraquinones, ascorbic acid, *p*-coumaric acid, cinnamic acid and pyrocatechol (Catalano *et al.*, 2024). The antibacterial activity of AVHE adds another important dimension to its therapeutic profile by reducing the risk of infection while promoting tissue regeneration.

The current study further examined the effect of AVHE on fibroblast proliferation and migration, two cellular events essential for wound healing. The proliferation and migration of fibroblasts has been embraced as a marker for evaluating the progress of wound healing. In our findings (Figures 5, 6), treatment with AVHE resulted in a significant fibroblast proliferation of 145.76% and a 57.03% wound closure rate. These findings suggest that AVHE considerably increases fibroblast proliferation and migration, which contributes to accelerated wound healing. The complex mechanism of cutaneous wound healing relies on a sequential interaction of multiple cells, growth factors, components of the extracellular matrix, and signaling pathways (Wilkinson & Hardman, 2020). Of particular importance, fibroblasts assume a pivotal role by undergoing proliferation and migration toward the wound site. Through direct or indirect interactions with a range of other cells, including keratinocytes, they contribute to the closure of the wound gap, thereby aiding the healing process (Smith & Rai, 2024). Based on the outcomes from our MTT and wound scratch assays, it is evident that AVHE exhibits an enhanced potential to promote proliferation and initiate migration in McCoy fibroblast cells, as compared to the control groups. Consistent with our findings, Negahdari *et al.* (2017), discovered that *A. vera* has the ability to enhance both fibroblast cell migration and proliferation.

The remarkable regenerative potential of Zebra fish makes them a suitable in vivo model for assessing the efficacy of therapeutics in tissue repair. Given the complicated interplay between

impaired connective tissue metabolism and delayed healing caused by diabetes, the exploration for effective wound-healing agents becomes necessary.

A. vera, enriched with a variety of bioactive compounds, has demonstrated multiple therapeutic actions, including anti-inflammatory, anti-oxidant, and collagen synthesis effects (Arafa *et al.*, 2025). These mechanisms are considered to mediate through the potential reduction of platelet aggregation and vasoconstriction. This in turn scavenges free radicals and increases collagen synthesis. Furthermore, a previous investigation also reported that *A. vera* expedites the healing of skin wounds in diabetic models by promoting fibroblast proliferation and collagen synthesis (Wang *et al.*, 2024). In this context, the observed regeneration effects of AVHE in Zebra fish model highlights the potential therapeutic applications of AVHE in managing diabetic wounds, highlighting the significance of exploring novel approaches to address the complexity of multifactorial challenges associated with wound healing in hyperglycemic conditions.

Conclusion

The current study demonstrated the potential of AVHE in accelerating wound healing, which might stem from its ability to regulate and synchronize different phases of wound healing, mitigate oxidative stress, prevent microbial infections, and exert hypoglycemic effects. Furthermore, our findings indicated the efficacy of AVHE in stabilizing the RBC membrane, implying its potential anti-inflammatory properties. This study offers significant scientific support for considering the topical application of AVHE as a possible therapeutic candidate and adjunct therapy for diabetic patients experiencing difficulties in wound healing.

Limitations and Future Scope

This study demonstrates the broad therapeutic potential of AVHE in diabetic wound healing, including anti-oxidant, anti-inflammatory, anti-diabetic, antibacterial, and regenerative properties. However, the findings are confined to in vitro and Zebra fish models, which may not fully capture the complexities of human wound healing, especially in diabetic conditions. Future research on fractionation and identifying a bioactive fraction with complete characterization of the phytochemicals would be interesting alongside elucidating the underlying molecular pathways and development of optimized formulations for therapeutic usage. Furthermore, exploring synergistic effects with other phytocompounds may improve its efficacy in chronic wound management.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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Abbreviations

BHT, Butylated hydroxytoluene; DM, Diabetes mellitus; DFU, Diabetic foot ulcers; *Aloe vera*, *A. vera*; AVHE, *Aloe vera* hydro-alcoholic extract; HCl, Hydrochloric acid; NaOH, Sodium hydroxide; $FeCl_3$, Ferric chloride solution; H_2SO_4, Sulfuric acid; FC, Folin-Ciocalteau; TPC, Total phenolic content; TFC, Total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2–2′-azino-di-(3-ethylbenzthiazoline sulfonate); DNSA, Dinitrosalicylic acid; PBS,

Phosphate Buffer Saline; DMEM, Dulbecco's Modified Eagle Medium; FBS, Fetal Bovine Serum; 2-NBDG, 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO, Dimethyl sulfoxide

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