INFORMATICS JOURNALS

Research Article

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/52816

eISSN: 2320-3358 | Vol 26(4), 2026 | pp. 991-1004

Evaluation of Wound Healing Activity of Polysaccharides of Morel Mushroom *Morchella esculenta*

Yamuna Vasudevan[1], Vineeta Debbie Nesam[1], Thiyanesh Vijay[1], Rakeshkumar Kesavaraman Rajaram[1], Vivek Kumar Dhiman[2], Sushmita Kalaichelvan[2] and Vijayarangan Sivasamy[1]*

[1]Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam - 603103, Tamil Nadu, India; drsvr1976@gmail.com

[2]Centre for Herbal Pharmacology and Environmental Sustainability, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam - 603103, Tamil Nadu, India

Abstract

Background: Recent attention is gaining towards polysaccharides derived from mushrooms like *Morchella esculenta* for their biocompatibility, antioxidant, and wound healing properties. It is necessary to promote alternatives for skin regeneration, as Chronic wounds remain a significant clinical challenge in healing. **Aim:** This research is aimed at evaluating the wound healing activity of polysaccharides extracted from *M. esculenta* through comprehensive *in vitro* and *in vivo* experimental models. **Methods:** Polysaccharides were extracted via a hot water–ethanol precipitation method. Cytotoxicity and fibroblast migration were evaluated in HaCaT cells and L929 fibroblasts using MTT and scratch assays. 36 Wistar albino rats were assigned to the excision wound model and treated topically with formulations containing 500 mg/kg and 1000 mg/kg *M. esculenta* polysaccharides for 28 days. Outcomes included wound contraction, epithelialization time, ELISA assays for hydroxyproline, catalase, TNF-α, IL-6, RT-PCR for VEGF-A, and histopathological and immunohistochemical analysis. The study was done after obtaining ethical clearance from the Institutional Animal Ethics Committee (IAEC 1 / Proposal: 156/A. Lr:118/ Dt: 20:02:2024) of Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu 6030103. **Results:** *M. esculenta*-derived polysaccharides significantly enhanced fibroblast migration at 12.5 µg/mL *in vitro*. In the *in vivo* model, the 1000 mg/kg dose demonstrated enhanced wound contraction. ELISA analysis showed increased hydroxyproline and catalase levels, along with reduced TNF-α and IL-6 concentrations. VEGF-A expression was significantly upregulated, indicating strong pro-angiogenic activity. Improved re-epithelialization, greater collagen deposition, and better tissue organisation in the treated groups compared to both control and standard drug-treated animals were observed in histopathological examinations. **Conclusion:** Multi-targeted wound-healing activity, such as fibroblast migration, reducing inflammation, and promoting angiogenesis were achieved by *M. esculenta*-derived polysaccharides. Although limited to animal models, these findings justify future translational studies

Major Findings: Polysaccharides from *Morchella esculenta* showed antioxidant and anti-inflammatory effects and improved fibroblast migration. High doses sped up wound healing similar to silver sulfadiazine. They lowered TNF-α and IL-6 and increased collagen formation.

Keywords: *Morchella esculenta,* Polysaccharide, Wound Healing

Abbreviations: c-DNA - complementary DNA, DMEM - Dulbecco's Modified Eagle Medium, DMSO - Dimethyl Sulfoxide, DPBS - Dulbecco's Phosphate Buffered Saline, EGF - Epidermal Growth Factor, FBS - Fetal Bovine Serum, H and E - Hematoxylin and Eosin, IAEC - Institutional Animal and Ethics Committee, M. e P - *Morchella esculenta* Polysaccharide, MT Masson's Trichrome, RNA - Ribonucleic Acid, RT-PCR - Reverse Transcription Polymerase Chain Reaction, SSD - Silver Sulfadiazine, VEGF-A - Vascular Endothelial Growth Factor – A.

(Article Chronicle: Article Received on: 28.10.2025; Revised on: 16.03.2026; Accepted on: 25.03.2026)

*Author for correspondence

1. Introduction

The physiological process that involves in restoration of tissue structure and function following an injury is wound healing[1]. There are various sequences involved in wound healing like "haemostasis, inflammation, proliferation, and remodelling"[2]. There are many cellular and molecular mediators involved in regulation of each phase ensuring rapid and efficient tissue repair. Modern medicine provides many synthetic and pharmacological substances, such as antiseptics, antibiotics, growth hormones, and silver-based topical formulations like silver sulfadiazine (SSD), to accelerate healing, prevent infection, and minimise scarring[3,4]. Even with the great efficacy of these therapies, there are certain drawbacks like high costs, prolonged healing in chronic wounds, risk of antibiotic resistance, allergic reactions, and cytotoxic effects[5]. Looking for the alternative therapy, herbal drugs have gained considerable attention for their biocompatibility, cost-effectiveness, and minimal adverse effects[6]. There are numerous bioactive compounds found in medicinal plants improving wound healing, such as flavonoids, alkaloids, tannins, and polysaccharides, which provides anti-inflammatory, antioxidant, antibacterial, and fibroblast-stimulating properties[7]. Also, these compounds promote angiogenesis, boost collagen synthesis, and assist re-epithelialization, all of which are essential for tissue regeneration. Use of these herbal medicines in wound management offers a holistic approach, particularly useful in resource-limited settings and for the management of chronic wounds when conventional treatments are insufficient.

In comparison with acute wounds, chronic wounds holds a considerable global health challenge, particularly in those with underlying conditions such as diabetes, infections, or immunocompromised individuals[8]. Though it is advanced, Modern medicines still face certain limitations, such as prolonged healing, infection susceptibility, high expenses, and inadequate bioactivity[9]. This has made interest in investigating natural bioactive compounds with intrinsic therapeutic qualities facilitating the wound healing process. Polysaccharides from alternative sources have gained considerable attention due to their enhanced biocompatibility, biodegradability, moisture-retention capabilities, and ability to modulate critical physiological processes such as inflammation and tissue regeneration[10,11].

It was found that polysaccharides derived from medicinal mushroom possessing antioxidant, immunomodulatory, and antibacterial activities, which play a vital role in various phases of wound healing[12]. *Morchella esculenta*, popularly known as the morel mushroom, is gaining attention for its nutritious and medicinal value[13]. On phytochemical analyses, it was found that *M. esculenta* was abundant in bioactive constituents such as polysaccharides, amino acids, vitamins, minerals, phenolic compounds and the extracted polysaccharides elicit several pharmacological effects, including antioxidant and anti-inflammatory properties, highlighting their usage for wound healing[14,15]. But there is a lack of valid scientific evidence in using *M. esculenta* polysaccharides in wound care, particularly through *in vivo* models.

The purpose of this study was to assess the wound healing performance of polysaccharides derived from *M. esculenta* using a standardised excision wound model in Wistar rats. A wide range of biological factors were looked at, including the pace of wound contraction, epithelialization, histological alterations and the expression of genetic markers linked to tissue regeneration. This research seeks to clarify the function of mushroom-derived polysaccharides in regenerative medicine and build a clinical foundation for the creation of safe, natural wound healing medicines. Results of this study may facilitate future research and substantially improve the growing field of mushroom-derived bioactives used in contemporary healthcare.

2. Materials and Methods

2.1 Materials

Dulbecco's modified Eagle medium (DMEM, Hyclone) was the culture medium, which was prepared in supplementation with 10% fetal bovine serum (FBS, Hyclone) and antibiotics (Penicillin 100 U/mL and Streptomycin 100 μg/mL, Gibco), Verso cDNA synthesis kit (ThermoFisher, USA).

2.2 Identification and Isolation of Polysaccharide Extract

The whole plant of *M. esculenta* was collected and authenticated. After cleansing, the mushrooms were shade-dried and ground using a mechanical grinder to obtain a fine powder. Approximately 1 kg of dried

mushroom powder was submerged in 4 L of methanol for two weeks at room temperature to remove pigments and other low-molecular-weight compounds, and the mixture was then filtered. The residues remaining on the filter paper were retained for polysaccharide extraction.

The hot water extraction method was used to extract polysaccharides. The residue was extracted with 10 L of distilled water (1:10 w/v) and heated at 90–95 °C for 3 hours with continuous stirring. The mixture was then cooled to room temperature and filtered to obtain the aqueous extract containing soluble polysaccharides.

The filtrate was subsequently mixed with 95% ethanol in a ratio of 1:3 (extract: ethanol) to precipitate polysaccharides and was kept at 4 °C overnight to ensure complete precipitation. The mixture was then centrifuged for 25 minutes at approximately 2500 rpm. Following centrifugation, the supernatant was carefully discarded, and the pellets of crude polysaccharides were separated, washed with ethanol, air-dried, and stored at below 0 °C until further use. The extraction yielded approximately 20–30 g of crude polysaccharide, corresponding to a 2–3% yield from 1 kg of dried mushroom powder.

2.3 Antioxidant Activity (DPPH Reduction Assay)

The antioxidant activity was performed using the "DPPH (2,2-diphenyl-1-picrylhydrazyl)" free radical scavenging assay. A mixture of 1 mL of 0.4% DPPH solution and 1 mL of the test sample was taken in a test tube. This reaction mixture was incubated for 30 mins in a dark place. After incubation, the absorbance was measured at 517 nm. "The radical scavenging activity was measured as a decrease in the absorbance of DPPH". The DPPH radical scavenging capacity was estimated from the difference in absorbance for the drug and the control blank. In this assay, the control typically shows higher absorbance at 517 nm. The data was expressed in percentage of DPPH scavenging. The IC50 (concentration of extracts resulting in 50 % reduction of the DPPH radical) values were calculated using linear regression analysis with GraphPad Prism software.

DPPH Scavenging activity (%) = [Abs of control – Abs of sample/Abs of control] x 100

The experiment was performed with ascorbic acid as a standard antioxidant compound, and the AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) was calculated for the test sample with the formula, (IC_{50} of Ascorbic acid / IC_{50} of test sample) x 100[16].

2.4 Anti-inflammatory Activity (Egg Albumin Denaturation Assay)

"The anti-inflammatory activity was evaluated using the Egg Albumin Denaturation Method. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of test sample. A similar volume of double-distilled water served as the control. The prepared solutions were incubated at 37±2°C for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm. Diclofenac sodium was used as a reference drug. The percentage inhibition of protein denaturation was calculated by using the following formula[17]:

% inhibition = 100 × [V t / V C - 1]

Where,

V t = absorbance of the test sample,

V c = absorbance of control".

2.5 Cell Culture

"HaCaT cells and L929 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS), 5 mM L-glutamine, and 50 U/ml each of penicillin and streptomycin"[18]. All cells in standard media were cultured in a humidified incubator with 95% air and 5% CO_2 at 37°C.

2.6 MTT Assay

The experiment was conducted using HaCaT cells exhibiting 70% confluence. The cells were subsequently treated with polysaccharide at several concentrations (0, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µg/mL) for a duration of 48 hours. The medium was changed once again for all groups, and 10 µl of MTT (5 mg/ml stock solution) was introduced, followed by an incubation period of 4 hours. The media was removed, and the formazan blue produced in the cells was solubilised using 150 µl of dimethyl sulfoxide (DMSO). The optical density was assessed at 590 nm. The % of cell viability and half maximum inhibitory concentration (IC50) value were calculated by using Eq. 1

$$Cell\ Viability(\%) = \frac{OD\ of\ the\ sample}{OD\ of\ the\ control\ group} * 100 \quad (1)$$

2.7 *In Vitro* Wound Healing Assay

The wound healing properties of polysaccharides were evaluated using *in vitro* cell migration tests on L929 cells using an established technique[19]. "2 x 10^5 cells/mL were inoculated onto 6-well plates and incubated overnight. The cells were subsequently rinsed with Dulbecco's Phosphate Buffered Saline (DPBS), and a scratch was created using a sterile 200µL pipette tip. The detached cells and cellular debris were eliminated by rinsing the cells with DPBS"[20]. The cells received treatment with 12.5 µg/mL of polysaccharide and 2 ng/mL of positive control (EGF), followed by a 48-hour incubation period. The untreated cells served as the negative control. An inverted microscope documented the cell movement and morphological alterations by means of a digital camera, which captured photos. The trials were conducted in triplicate (n = 3). The width of the scratch and wound closure was assessed using ImageJ software. The wound closure % was calculated by using Eq. 2

$$Wound\ Closure(\%) = \frac{Area_{t0} Area_{t48}}{Area_{t0}} * 100 \quad (2)$$

Where $Area_{t0}$ is the area at t = 0 h, and $area_{t48}$ is the area at t = 48 h.

2.8 *In Vivo* Wound Healing Assessment

2.8.1 Study Design and Grouping

This study was initiated following approval by the Institutional Animal and Ethics Committee (IAEC 1 / Proposal: 156/A. Lr:118/ Dt: 20:02:2024). The study was carried out using 42 healthy Wistar albino rats of either sex, where each rat weighed between 200 to 250 grams. The rats were acquired from a verified vendor and maintained under conventional laboratory settings, comprising a 12-hour light/dark cycle, regulated temperature (22 ± 2°C), and relative humidity (55 ± 5%). The rats were acclimated to the experimental setting one week before the commencement of the experiment. Throughout the trial, the rats had unrestricted access to a regular pellet meal and clean drinking water.

2.8.2 Acute Dermal Toxicity

Based upon the OECD guidelines 402, 6 female rats were randomly selected, and 24 hours before the study, 10% of the body surface area fur was shaved from the dorsal area of the trunk and 200, 1000, and 2000 mg/kg of extract formulations were uniformly applied over the shaved area using a gauze dressing for 24 hours. Rats were observed every day over a period of 14 days in sight of spotting any adverse skin reactions and mortality, and the rats were sacrificed using an overdose of halothane[21].

2.8.3 Excision Wound Model

After 7 days of acclimatization, the rats were divided into 4 groups containing 6 rats in control group (group I) and 10 rats in each treatment Group (Group II, Group III, Group IV) where the Group I (control), Group II received (SSD), group III received (500 mg/k *M. e* P) and group IV received (1000 mg/k *M. e* P). "Anaesthesia with intraperitoneal injection of Ketamine (80 mg/kg) and Xylazine (13 mg/kg) was used on both female and male Wistar rats, and the dorsum of the rats was shaved, a full-thickness 10 mm skin wound was created and treated using the standard and test drugs respectively"[20]. An ointment base was prepared by incorporating *M. esculenta*-derived polysaccharide at a concentration of 5% w/w into petroleum jelly, and it was applied over the wound for 28 days. The wound dimensions were measured and photographed at baseline, 7th, 14th and 28th days. The wound contraction (%) was calculated according to equation (3). Rats were sacrificed using an overdose of halothane, and tissues were taken on the 7^{th}, 14^{th} and 28^{th} day.

$$Wound\ Contraction(\%) = \left(\frac{A_0 - A_n}{A_0}\right) * 100 \quad (3)$$

where A_0 was the original wound area on day 0, and A_n was the wound area on day 7, 14, and 28 respectively[22].

2.8.4 Real-time PCR Analysis

RNA extraction from the skin tissue sample was done using the TRIzol technique following the manufacturer's procedure. Following homogenization using the QIAshredder spin column, the material was transferred to the QIAamp spin column. Total RNA adheres to the QIAamp membrane while impurities

are removed, resulting in the elution of pure RNA in 30–100 µl of RNase-free water (included in the kit) for immediate use in cDNA synthesis. Subsequent cDNA synthesis conversion was conducted utilising a two-step Verso cDNA synthesis kit (ThermoFisher, USA) in accordance with the previously delineated approach[23]. "PCR amplification was conducted in final quantities of 25 µl, including 5 µl of cDNA template, 0.2 µM of each primer (VEGF-A and β-Actin), and 12.5 µl of SybrGreen Master Mix (Applied Biosystems). All amplifications were conducted on optical-grade 96-well plates utilising an A.B.I. Prism 5700 sequence detection system (Applied Biosystems), commencing with an initial step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 30 seconds"[24]. The device automatically calculated the CT. Every sample was examined in duplicate. Forward and reverse primer sequences are mentioned in Table 1 for VEGF-A and β-Actin (Table 1).

2.8.5 Histological Evaluation

Histopathological evaluation was performed to examine the wound healing and structural alterations at the wound site. Skin samples were obtained at specified intervals (7^{th},14^{th} and 28^{th} day), fixed in 10% neutral-buffered formalin for 24–48 hours, dehydrated using a graded ethanol series, cleaned with xylene, and embedded in paraffin. Thin tissue slices of 5 µm in thickness were procured with a rotary microtome and affixed to glass slides. The sections were subsequently fixed with "Hematoxylin and Eosin (H and E) and Masson's Trichrome (MT)" methods of staining. A light microscope was used to analyse stained slides. Histological evaluation focused on tissue regeneration, cellular composition, collagen deposition and extracellular matrix, to determine the progression of wound healing among different treatment groups.

2.9 Statistical Analysis

Data were provided as "mean ± standard deviation. Statistical analyses were done utilising Graph Pad Prism 7.0 software (La Jolla, CA, USA). One-way ANOVA followed by Tukey's multiple comparison test were used for conducting Group comparisons. $p < 0.05$ at all instances were deemed to be statistically significant.

3. Result

3.1 Antioxidant Activity

The antioxidant activity of *Morchella esculenta* polysaccharides was evaluated using the DPPH radical scavenging assay Figures 1A, 1B, 1C and 1D. As shown in Figure 1A, the polysaccharide extract exhibited a concentration-dependent increase in scavenging activity. At 100, 200, 400, 600, 800, and 1000 µg/mL of the polysaccharide extract, the scavenging effects were 63.45%, 69.60%, 72.68%, 82.90%, and 87.26%, respectively, and the standard antioxidant ascorbic acid (1.5–2.5 µg/mL) exhibited scavenging activity ranging from 61.20% to 70.55%. Dose-response curve analysis revealed that the IC_{50} value of *M. esculenta* polysaccharides was 458.4 µg/mL Figure 1B, whereas ascorbic acid showed a much lower IC_{50} of 0.1864 µg/mL Figure 1C. The calculated "AEAC (Ascorbic Acid Equivalent Antioxidant Capacity)" of the polysaccharides was 0.0407%.

3.2 Anti-inflammatory Activity

Morchella esculenta polysaccharides' anti-inflammatory activity was assessed with the employment of the egg albumin denaturation method and compared to the standard drug diclofenac Figure 2. At 100 µg/mL, the extract showed 27% inhibition, which was slightly lower than diclofenac (33%), showing no statistically significant difference. At 200 and 300 µg/mL, the polysaccharides exhibited 53% and 67% inhibition, respectively, which was significantly lower than diclofenac (87% and 87%) with ****p value < 0.0001. However, at higher concentrations of 400 and 500 µg/mL, the polysaccharides displayed 86% and 93% inhibition, which were comparable to diclofenac (92% and 99%), showing no significant differences.

Table 1. VEGF-A and β-Actin primer sequence

	Forward	Reverse	Amplicon Size
VEGF-A	5′-AGGGCAGAATCATCACGAAGT-3′	5′-AGGGTCTCGATTGGATGGCA-3′	~150 bp
β-Actin	5′-AGAGCTACGAGCTGCCTGAC-3′	5′-AGCACTGTGTTGGCGTACAG-3′	~150 bp

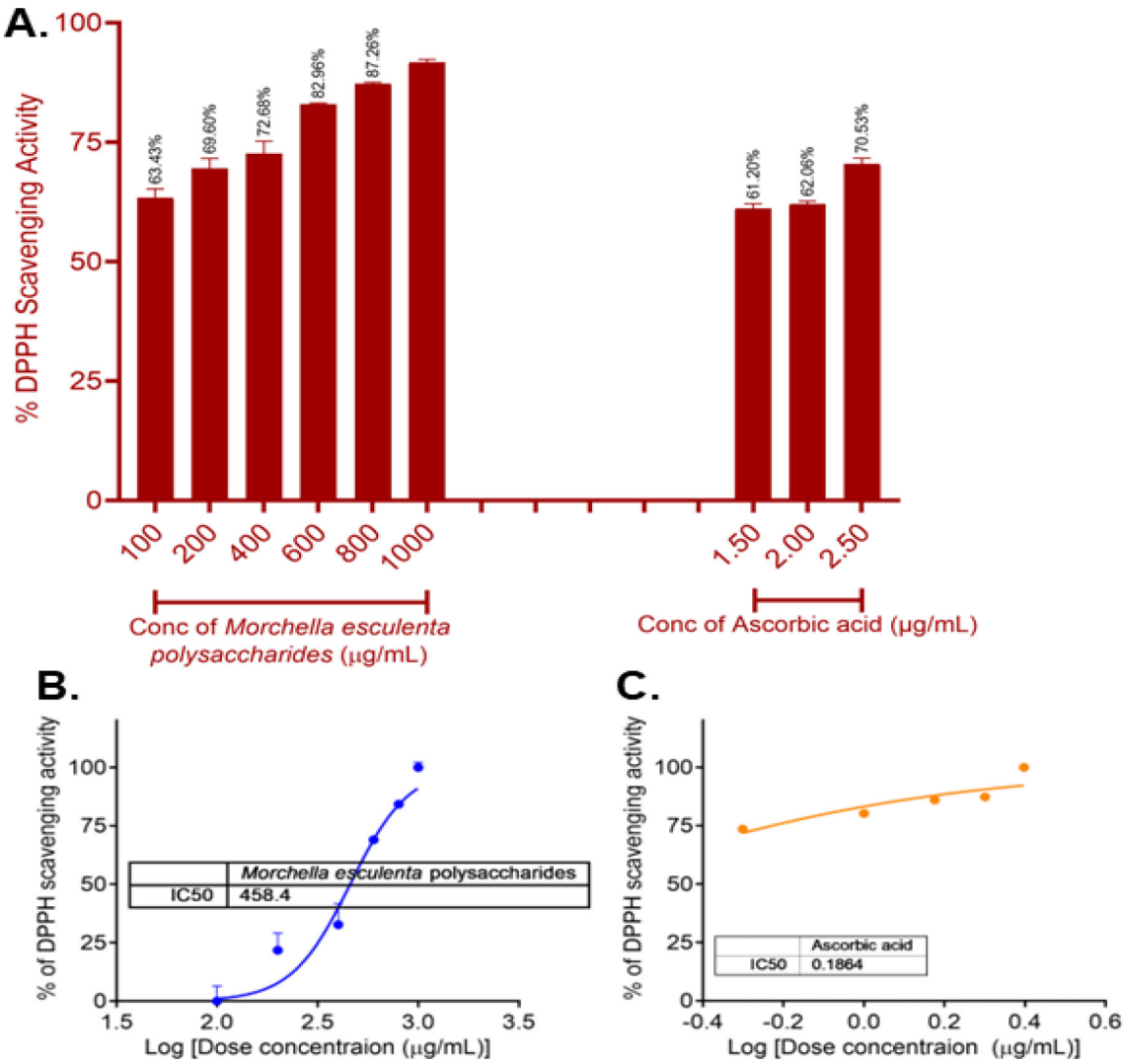


Figure 1. *In vitro* antioxidant activity of *Morchella esculenta* polysaccharides and Ascorbic acid. **A.** Dose-dependent DPPH radical scavenging activity (%). **B.** IC_{50} value of *M. esculenta* polysaccharides using DPPH assay. **C.** IC_{50} of Ascorbic acid extract using DPPH assay.

3.3 MTT Assay

MTT results indicated that at a 100 µg/mL concentration of polysaccharide extract, cell viability decreased markedly to 24.83%, signifying substantial cytotoxicity (Figures 3A, 3B, 3C and 3D). "As the concentration decreased, cell viability progressively improved: 30.31% at 50 µg/mL, 42.97% at 25 µg/mL, and 47.57% at 12.5 µg/mL, corresponding to the IC_{50} value (concentration at which 50% of cell viability is suppressed)". Moderate cytotoxicity was noted at 6.25 µg/mL, resulting in 71.83% cell viability, but lower doses of 3.125 µg/mL and 1.56 µg/mL exhibited 79.08% and 91.18% of cell viability, respectively. These findings validate that the polysaccharide produced from *M. esculenta* demonstrates a dose-dependent cytotoxic impact on L929 cells, with an IC_{50} of 12.5 µg/mL, underscoring its potential as a bioactive molecule with selective cytotoxicity.

3.4 *In Vitro* Wound Healing Assay

The *in vitro* scratch wound healing assay, which utilised L929 fibroblast cells, revealed that the *M. e* P at 12.5 µg/mL concentration significantly promoted wound closure over 48 hours compared to the untreated control (Figures 3C and 3D). The initial wound area was standardised to 100% across all experimental groups at 0 hours. The control group exhibited minimal wound closure, retaining 99.35% of the wound area, indicating negligible cell

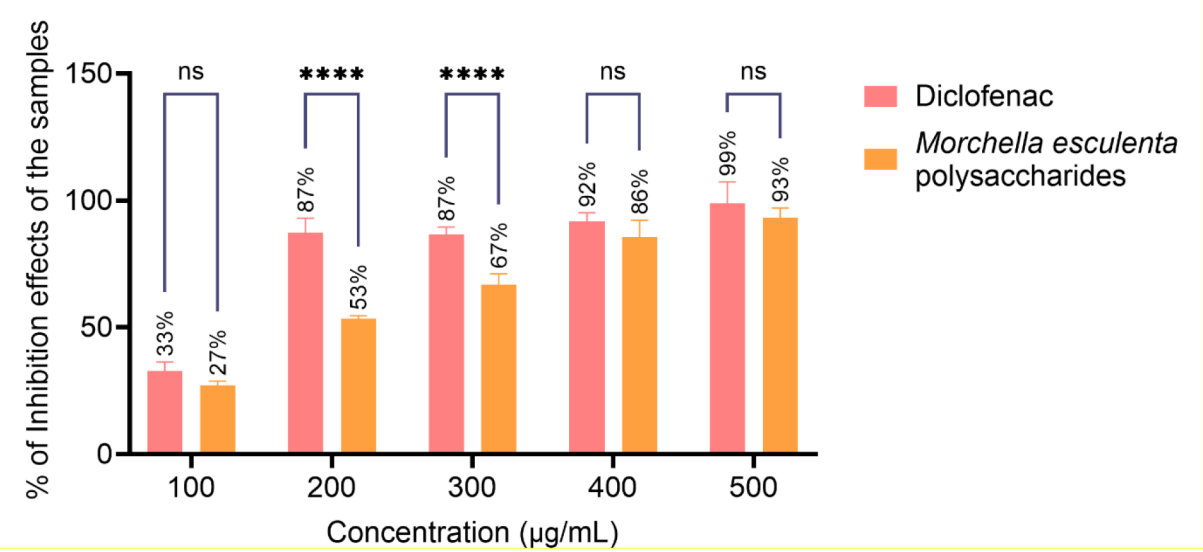


Figure 2. *In vitro* anti-inflammatory activity of *M. esculenta* polysaccharides compared with diclofenac using egg albumin assay.

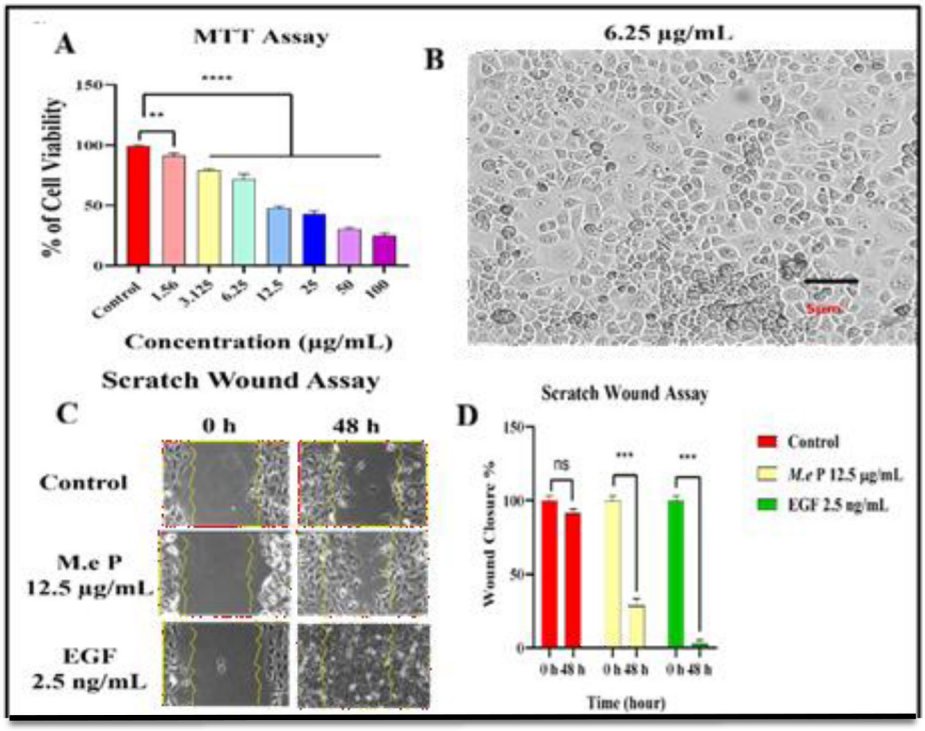


Figure 3. **(A).** MTT assay against HaCaT cell lines; **(B).** pictorial representation of HaCaT cell lines at 6.25 µg/mL concentration; **(C).** pictorial representation for scratch wound assay at 0 and 48 h against L929 cell lines and **(D).** % of wound closure graph.

migration after 48 hours. In contrast, the group treated with *M. e* P 12.5 µg/mL showed a reduction in wound area, averaging around 28.8%, signifying enhanced fibroblast migration and proliferation. The wound area is reduced to approximately 2.9% in the positive control, treated with epidermal growth factor (EGF) at 2.5 ng/mL. These results confirm that *M. eP* enhances fibroblast motility and contributes to wound closure.

3.5 Acute Dermal Toxicity

The "acute dermal toxicity test performed on female Wistar rats showed no signs of systemic toxicity and negative skin responses after polysaccharide extract at doses of 200, 1000, and 2000 mg/kg were applied topically". As observed over 14 days, all rats were healthy and displayed typical behaviour, food habits, and physical activity. No erythema, oedema, or ulcerative dermal lesions were observed at the application site in any extract-treated group. Furthermore, there were no occurrences of morbidity or fatality. The lack of visible clinical symptoms or systemic toxicity at the maximum tested dose (2000 mg/kg) of *M.e.P* suggests that the formulation is well-tolerated and safe for dermal application in this study.

3.6 *In Vivo* Wound Healing

The *in vivo* wound healing study demonstrated a progressive, time-dependent increase in wound contraction among all experimental groups, with the SSD and both dosages of *M. e* P exhibiting markedly superior wound closure relative to the untreated control group (Figures 4A and 4C). On Day 7, rats treated with SSD showed a statistically significant increase in wound contraction compared to the control group ($p < 0.0001$), with a mean difference of 11.45% (95% CI: 5.83–17.07). SSD treatment demonstrated greater efficacy compared to the low dose *M. e* P group (500 mg/kg), with a mean difference of 6.36% ($p = 0.0226$). Conversely, no significant difference was noted between SSD and the high dose *M. e* P group (1000 mg/kg), suggesting that the high dose produced a healing response statistically similar to that of SSD ($p = 0.6807$) (Figure 4A). On Day 14, the wound healing effect of SSD remained superior, exhibiting significant differences when compared with the control group ($p < 0.0001$; mean difference: 28.74%, 95% CI: 23.12–34.36) as well as with the 500 mg/kg M.e.P -treated group ($p < 0.0001$; mean difference: 12.34%, 95% CI: 6.72–17.96). Nonetheless, no significant difference was seen between the SSD and the 1000 mg/kg *M. e* P group ($p = 0.3592$), further suggesting that the high-dose extract had a wound healing effect statistically comparable to the conventional therapy (Figure. 4B). On Day 28, SSD exhibited significant improvement in wound contraction relative to the control group ($p < 0.0001$; mean difference = 38.97%, 95% CI: 33.35 to 44.59) and the 500 mg/kg *M. e* P group ($p < 0.0001$; mean difference = 14.93%, 95% CI: 9.314 to 20.55). A statistically significant difference was noted between SSD and the high dose *M. e* P group ($p = 0.0108$; mean difference = 7.00%, 95% CI: 1.384 to 12.62), indicating that *M. e* P extract showed enhanced wound healing similar to the standard drug. SSD maintained a marginally superior efficacy at the final observation point. The results demonstrate a dose-dependent impact of *M. esculenta* extract on wound healing, with the high dose (1000 mg/kg) of *M. e* P extract representing wound contraction equivalent to SSD at day 7 and 14, however slightly less effective at day 28th.

3.7 Histopathological Examination of Wound Tissue

Histopathological analysis of skin tissues stained with H and E and Masson trichome on Days 7, 14, and 28 (Figures 4D and 4E) revealed pronounced differences in wound healing progression across the treatment groups. The control group showed ulceration of the epidermis accompanied by granulation tissue in the dermis, marked by infiltration of the inflammatory cells, fibroblasts, and neovascularisation, but the surrounding dermis maintained typical appendageal structures on day 7. The standard drug exhibited healing response like both the high dose (1000 mg/kg *M. e* P) and low dose (500 mg/kg *M. e* P), revealing significant epithelial regeneration and mature scar tissue formation characterised by fibroblast and collagen deposition, alongside the preservation of normal dermal appendages. MT staining showed the existence of irregular, thin, and scattered collagen fibres in the control group, while the treatment groups (SSD, low, and high dose) exhibited mature scar tissue characterised by regularly arranged thin collagen fibres. The control group continued to display ulceration accompanied by persistent granulation tissue and neovascular growth, signifying delayed wound healing by day 14. The low dose group exhibited only persistent granulation tissue and angiogenesis, indicating inadequate effectiveness at this concentration. In contrast, the high dose *M. e P* group displayed comparable histological characteristics to the SSD-treated group, characterised by full epithelial regeneration and mature scar tissue with substantial fibroblast and collagen accumulation. MT staining corroborated these findings: both the control and

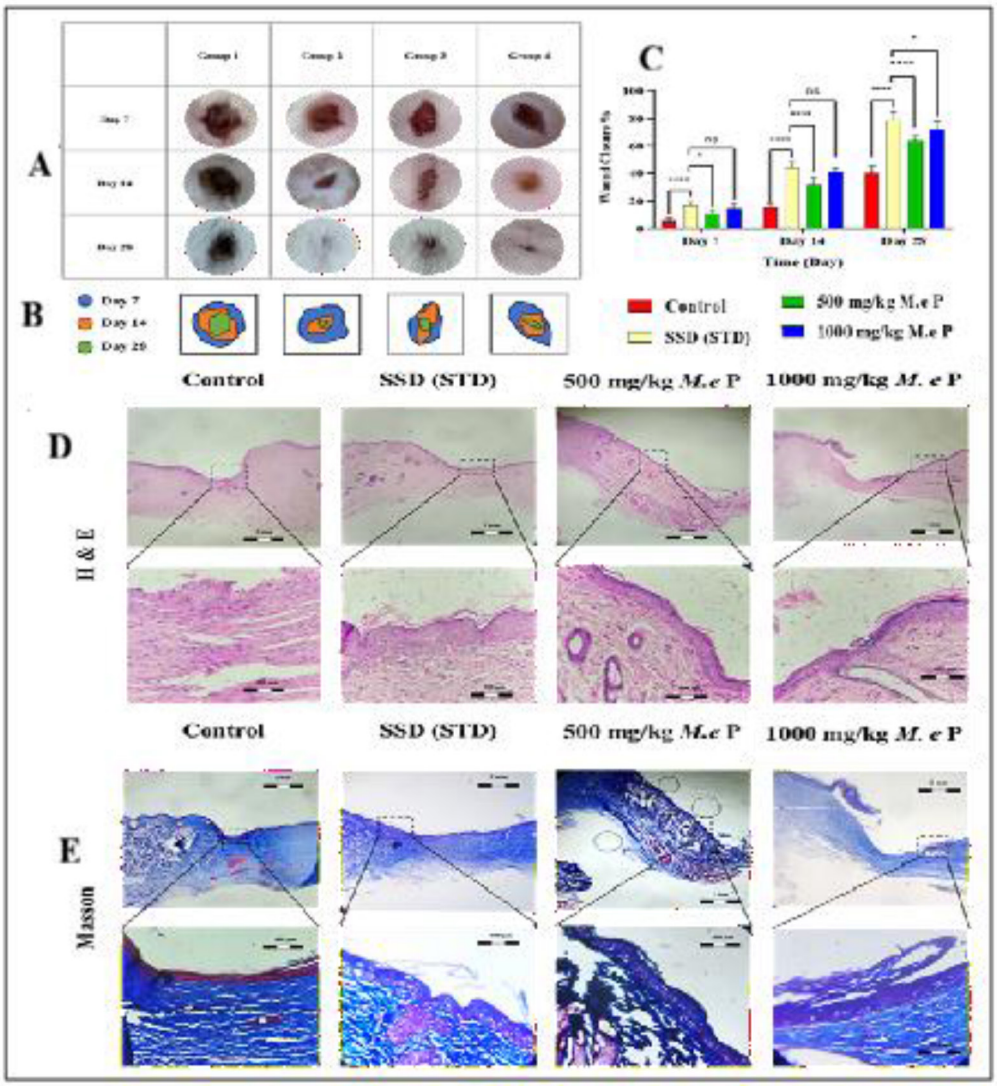


Figure 4. Assessment of *in-vivo* wound healing. **(A).** Representative photographs of skin wounds group I, group II, group III and group IV; **(B).** schematic illustration depicting wound closure at different time points and **(C).** wound closure rate of each group at 7th, 14th and 28th days; **(D).** H and E and **(E).** Masson trichome staining of the wounds in multiple groups on day 28.

low-dose groups had uneven and attenuated collagen fibres, signifying persistent inflammation and nascent scar formation. Well-structured, uniformly placed mature collagen fibres indicative of extensive wound remodeling was observed in both the high dose and SSD groups. The control group exhibited incomplete healing, indicated by ongoing ulceration, inflammatory infiltrates, fibroblasts, and vascular growth in the dermis by day 28. Tissue regeneration, which was comparable to the SSD-treated group, was observed in both treatment groups (low and high dose of *M. e P*), viz., re-epithelialized epidermis, mature scar tissue abundant in fibroblasts and collagen, and preserved appendageal structures in the dermis. The existence of mature scar architecture is characterised by well-organised collagen deposition over the treatment groups, but the control group exhibited uneven immature collagen deposition on day 28. High doses of *M. esculenta* polysaccharides showed wound healing efficacy similar to SSD, whereas low doses exhibited a delayed but ultimately beneficial response, highlighting a dose-dependent healing impact.

3.8 ELISA Analysis

Hydroxyproline, TNF-α, IL-6, and catalase levels were assessed in the wound healing model across the control,

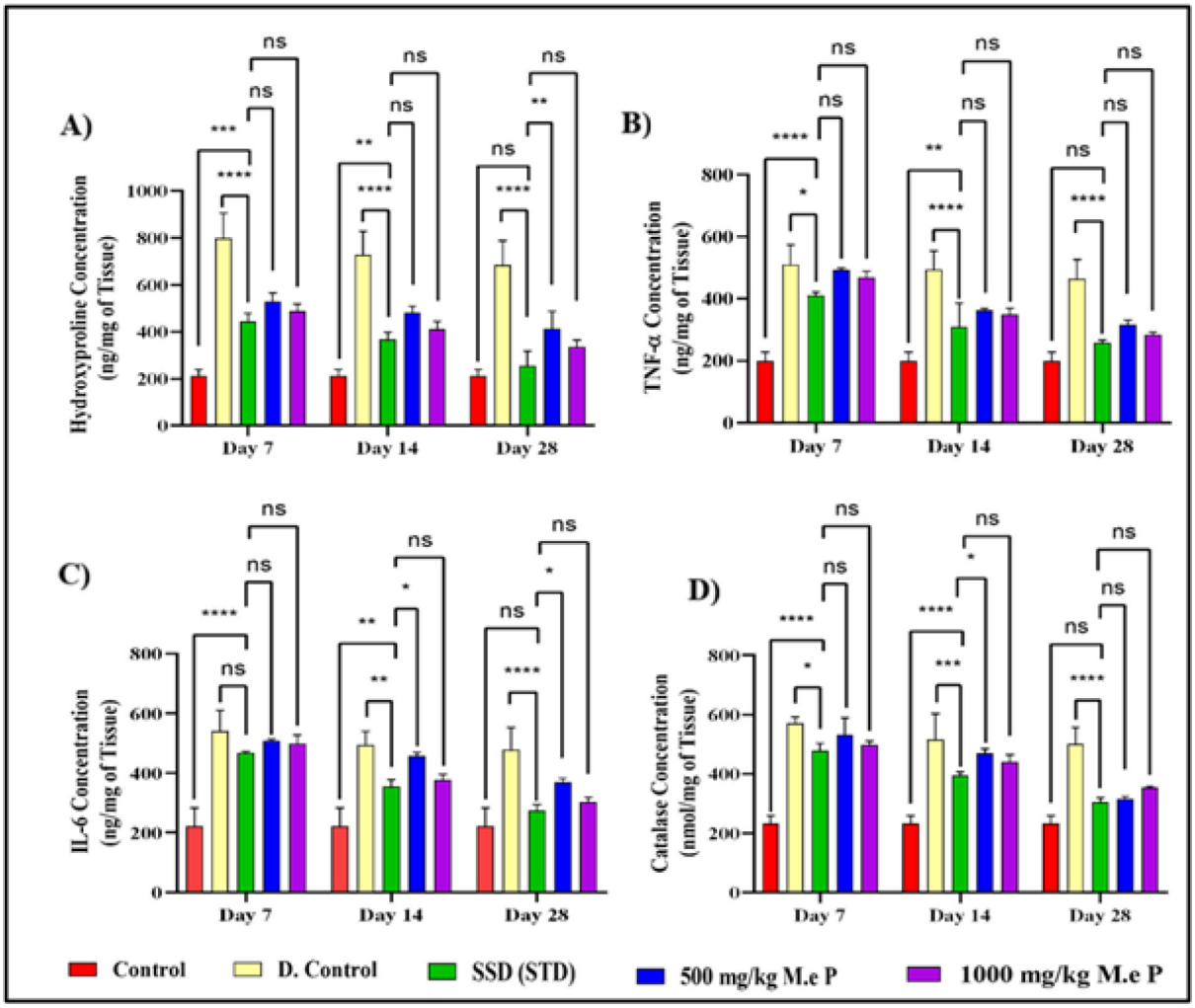


Figure 5. ELISA analysis of hydroxyproline **(A);** TNF- α **(B);** IL-6 **(C)** and catalase **(D)**.

standard (SSD), and *M. esculenta* polysaccharide-treated groups (500 mg/kg and 1000 mg/kg) on Days 7, 14, and 28 (Figure 5), using ELISA. Hydroxyproline, a marker of collagen deposition, was significantly elevated in all treated groups compared to the untreated control, with the 500 mg/kg and 1000 mg/kg doses (of *M. esculenta*) showing peak levels at Day 7 (529 ± 36.8 ng/mL and 488 ± 30.9 ng/mL, respectively), and a gradual decline by Day 28 (413 ± 75 and 336 ± 29.7 ng/mL), indicating active tissue remodeling. Pro-inflammatory cytokines TNF-α and IL-6 were substantially higher in the untreated group, with TNF-α reaching 510 ± 64.5 ng/mL and IL-6 at 541 ± 69 ng/mL on Day 7. Both cytokines progressively decreased in treated groups, with the 1000 mg/kg dose showing greater suppression by Day 28 (TNF-α: 284 ± 8 ng/mL; IL-6: 302 ± 16.1 ng/mL), reflecting anti-inflammatory activity. Catalase levels, an indicator of antioxidant defence, were lower in the control group across all time points but significantly restored in the *M. esculenta* polysaccharide extract-treated groups, particularly on Day 7 in the 500 mg/kg (532 ± 57.9 nmol/mg) and 1000 mg/kg (497 ± 14.2 nmol/mg) groups. These findings demonstrate that *M. esculenta* polysaccharide enhances wound healing by promoting collagen synthesis, reducing inflammation, and restoring oxidative balance in a dose- and time-dependent manner.

3.9 Gene Expression Analysis

The expression of the VEGF-A gene was assessed by RT-PCR to evaluate angiogenic activity during the wound healing process across control, standard (SSD), and *M. esculenta* polysaccharide-treated groups at 500 mg/kg and 1000 mg/kg doses (Figure 6 A). In the

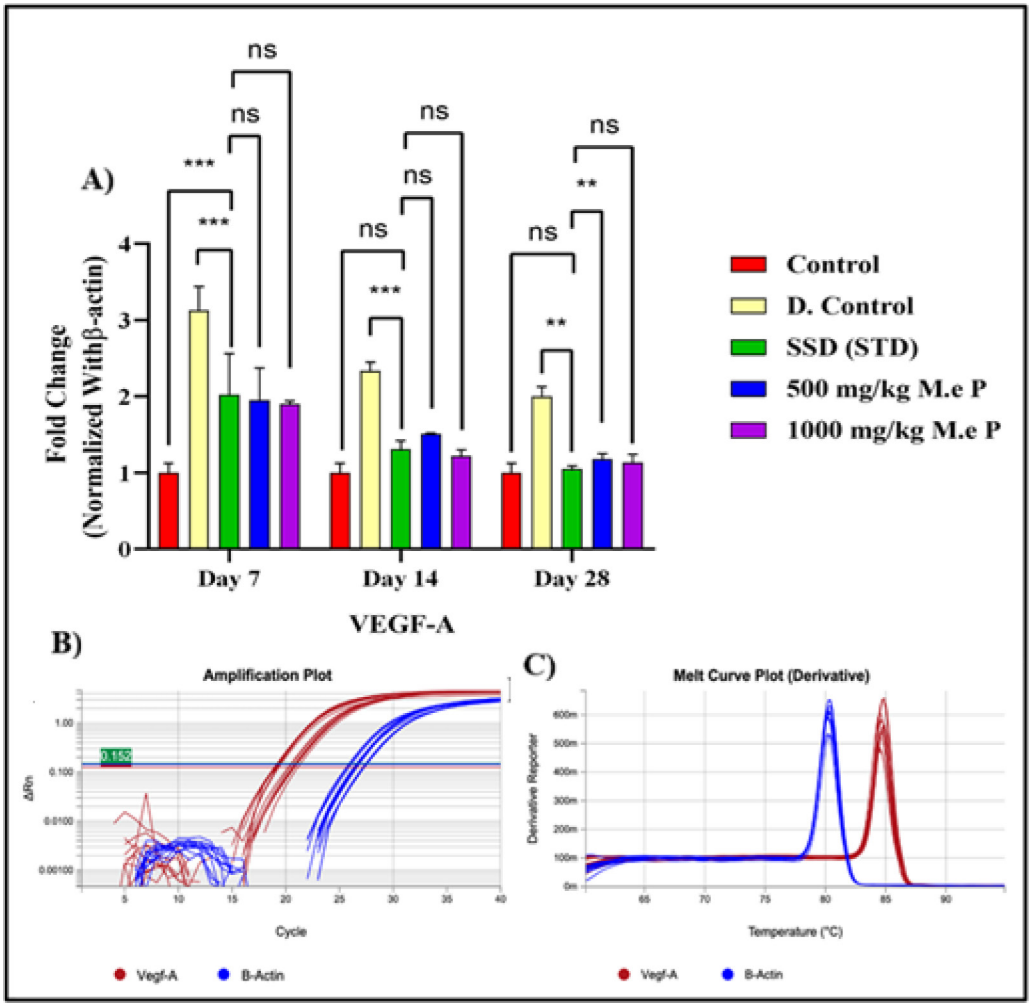


Figure 6. Gene expression of VEGF-A treated with different groups **(A)**, amplification plot **(B)** and melt curve plot **(C)**.

untreated control group, VEGF-A expression remained consistently low across all time points (1.00-fold). A significant upregulation of VEGF-A was observed in the SSD-treated group, peaking at Day 7 with a 2.02 ± 0.54-fold increase and gradually decreasing to 1.05 ± 0.04-fold by Day 28. Treatment with *M. esculenta* polysaccharide at 500 mg/kg also demonstrated elevated VEGF-A expression, reaching 1.95 ± 0.42-fold at Day 7 and declining to 1.18 ± 0.07 by Day 28. Similarly, the high dose of *M.e.P*(1000 mg/kg) induced a 1.90 ± 0.04-fold increase in VEGF_A expression at Day 7, followed by a reduction to 1.14 ± 0.10-fold by Day 28. These findings show that there was dose and time-dependent healing on *M. esculenta* polysaccharide treatment, by improving VEGF-A expression. The amplification plot and melt curve analysis of VEGF-A and B-Actin genes were used to identify the specificity and efficiency of the RT-PCR assay. The melt curve analysis further determined the specificity of the PCR products, showing distinct and sharp single peaks for both VEGF-A and B-Actin at different melting temperatures, with no evidence of nonspecific products or primer-dimer formation (Figure 6 C). In the amplification plot, the VEGF-A gene (represented in red) showed earlier cycle threshold (Ct) values compared to the B-Actin housekeeping gene (in blue), indicating a higher relative expression of VEGF-A in treated samples. The amplification curves were smooth and consistent, reflecting uniform and exponential amplification without anomalies (Figure 6 B).

4. Discussion

The present study demonstrated the greater wound healing activity of *Morchella esculenta* through *in vitro* and *in vivo* results. There are certain therapeutic benefits

of *M.e.P* for chronic wound includes antioxidants, anti-inflammatory, pro-angiogenic, and collagen-stimulating properties. Together, these properties speed up the inflammation, tissue proliferationand remodeling[12]. A dose-dependent cytotoxicity was observed with *M. esculenta* polysaccharides, with an IC_{50} value of 12.5 µg/mL on the MTT assay. Badshah *et al.* demonstrated similar findings that polysaccharides from *M. esculenta* enhanced cell adhesion and viability, while exhibiting cytotoxic effects at higher concentrations[25]. Teymoorian *et al.*, who done study on polysaccharides of *Trametes versicolor*, a non-edible fungus, demonstrated a dose-dependent cytotoxicity with acceptable biocompatibility, proving the presence of wound healing properties in polysaccharides derived from mushrooms[26]. The scratch assay done in this study shows improvement in fibroblast migration and wound closure on 12.5 µg/mL of *M. esculenta* polysaccharides in comparison with untreated controls. Iosageanu *et al.* did a study on polysaccharides obtained from *Aloe vera*, found that there was *in vitro* facilitation of fibroblast migration, supporting the current study[19]. In an identical study, Zhu *et al.* demonstrated that *Zizania latifolia* polysaccharides improved fibroblast proliferation and migration[27].

The excision wound model in the present study shows a dose-dependent enhancement with 1000 mg/kg of *M. esculenta* polysaccharides like SSD. Teymoorian *et al.*,[26] demonstrated that *T. versicolor* polysaccharides facilitated wound closure in rats after 14 days, similar to the improved healing shown in our high-dose cohort. Similarly, Zhu *et al.*,[27] demonstrated that *Z. latifolia* polysaccharides markedly improved wound closure and decreased inflammatory cytokines, similar to our dose-dependent findings on wound contraction. Yang *et al.* (2021)[5], in their study, highlighted that polysaccharides improve wound contraction through fibroblast activation and extracellular matrix remodelling, supporting our findings.

In our study, the histopathological analysis demonstrated improved epithelium regeneration, collagen deposition, and mature scar tissue in the treated groups, especially at the 1000 mg/kg dosage form. Al-Hammood *et al.*,[28] indicated that a tri-layer scaffold incorporating sulfated polysaccharides facilitated angiogenesis and collagen maturation, confirming our findings of structured collagen deposition in Masson's trichrome-stained sections. Assar *et al.*,[23] similarly demonstrated that liquorice extract improved epithelial regeneration and collagen fibre arrangement in injured tissues. Petrović *et al.*,[29] also observed improved epidermal regeneration and fibroblast accumulation in wounds treated with *Cerioporus squamosus* polysaccharide formulations, confirming our histological findings.

ELISA results indicated increased hydroxyproline levels, reduced TNF-α and IL-6, and improved catalase activity in polysaccharide-treated wounds, signifying collagen formation, anti-inflammatory effects, and antioxidant restoration. Zhu *et al.*,[27] similarly observed reduced levels of TNF-α and IL-6 in wounds treated with polysaccharides, supporting our anti-inflammatory findings. Falanga *et al.*,[8] emphasised that the inhibition of pro-inflammatory cytokines is essential for the shift of wounds into proliferative phases, confirming our findings. Moreover, Assar *et al.*,[23] similarly reported that treatment with plant polysaccharides increased antioxidant enzymes, such as catalase, which is in concordance with our findings.

VEGF-A expressions significantly increased in our study during the initial phase in polysaccharide-treated groups, promoting angiogenesis, before returning to baseline by day 28. Assar *et al.*,[23] documented a comparable early increase of VEGF-A during wound healing using liquorice extract, indicating increased angiogenesis. Al-Hammood *et al.*,[28] showed that deferoxamine-loaded scaffolds elicited robust VEGF-mediated angiogenesis, corroborating our observations of pro-angiogenic action. Petrović *et al.*,[29] also revealed that polysaccharide formulations obtained from mushrooms increased VEGF expression, confirming our findings on angiogenic stimulation. We demonstrated biocompatibility and no adverse effects in the acute dermal toxicity study, which further validates the polysaccharides for therapeutic use. This work provides significant preclinical data supporting the application of *M. esculenta*-derived polysaccharides as a bioactive agent in wound care. These findings require more research into formulation optimisation, molecular analyses and clinical validation to convert these discoveries into therapeutic solutions for human use.

5. Conclusion

Polysaccharides derived from *M. esculenta* showed great potential for wound healing based on the *in vitro*

and *in vivo* results of our study. The high dose (1000 mg/kg) of the *M. eP* demonstrated therapeutic effectiveness like that of the standard treatment, silver sulfadiazine, as shown by enhanced wound contraction, epithelium regeneration, and ordered collagen deposition. The treatment promoted fibroblast migration, stimulated collagen deposition, exhibited potent antioxidant and anti-inflammatory activity, and upregulated angiogenic factors such as VEGF-A. Histopathological assessments and ELISA substantiated these results, emphasising the dose-dependent efficacy of the extract. These results confirm the use of *M. esculenta*-derived polysaccharides as a safer alternative, compatible, and effective natural remedy for wound treatment, with encouraging prospects for future advancements.

6. Ethical Approval

The Research was done after getting ethics approval from the Institutional Animal Ethics Committee (IAEC 1 / Proposal: 156/A. Lr:118/ Dt: 20:02:2024) of Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu 6030103.

7. References

1. Rodrigues M, *et al.* Wound healing: A cellular perspective. Physiol Rev. 2019 Jan 1; 99(1):665-706. doi: 10.1152/physrev. 00067.2017
2. Landén NX, D. Li, and M. Ståhle, Transition from inflammation to proliferation: a critical step during wound healing. Cell. Mol. Life Sci. 2016; 73(20):3861-3885. https://doi.org/10.1007/s00018-016-2268-0
3. Vishwanath N, *et al.* Silver as an antibiotic-independent antimicrobial: review of current formulations and clinical relevance. Surgical Infections. 2022; 23(9):769-780. https://doi.org/10.1089/sur.2022.229
4. Chiu TW and Burd A. Key topics in plastic and reconstructive surgery. 2005: CRC Press. https://doi.org/10.1201/b14477
5. Yang F, *et al.* The biological processes during wound healing. Regenerative medicine. 2021; 16(4):373-390. https://doi.org/10.2217/rme-2020-0066
6. Ahmed SN, *et al.* Herbal drugs: safety, cost-effectiveness, regulation, current trends, and future directions, in bioprospecting of tropical medicinal plants. Springer. 2023; 1479-1493. https://doi.org/10.1007/978-3-031-28780-0_62
7. Annadurai DP, *et al.* Evaluation of antibacterial, antioxidant, and anticataleptic activity on methanol seed extract of *cannabis sativa*: an *in vitro* and *in vivo* study. J. Nat. Rem. 2024; 24(12):2713–2725. https://doi.org/10.18311/jnr/2024/44434
8. Falanga V, *et al.* Chronic wounds. Nature Reviews Disease Primers. 2022; 8(1):50. https://doi.org/10.1038/s41572-022-00377-3
9. Pathak D and Mazumder A. A critical overview of the challenging roles of medicinal plants in the improvement of wound healing technology. DARU J. Pharm. Sci. 2024; 32(1):379-419. https://doi.org/10.1007/s40199-023-00502-x
10. Sharma S and Bhende M. An overview: non-toxic and eco-friendly polysaccharides-its classification, properties, and diverse applications. Polymer Bulletin. 2024; 81(14):12383-12429. https://doi.org/10.1007/s00289-024-05307-9
11. Tunçer S. Biopolysaccharides: properties and applications. Polysaccharides: properties and applications. 2021; 95-134. https://doi.org/10.1002/9781119711414.ch6
12. Friedman M. Mushroom polysaccharides: chemistry and antiobesity, antidiabetes, anticancer, and antibiotic properties in cells, rodents, and humans. Foods. 2016; 5(4):80. https://doi.org/10.3390/foods5040080
13. Sunil C and Xu B. Mycochemical profile and health-promoting effects of morel mushroom *Morchella esculenta* (L.)-a review. Food Research International. 2022; 159:111571. https://doi.org/10.1016/j.foodres.2022.111571
14. Wu H, *et al.* Recent Advances in the Bioactive Ingredients of *Morchella esculenta*. Appl. Biochem. Biotechnol. 2021; 193(12):4197-4213. https://doi.org/10.1007/s12010-021-03670-1
15. Ding X, *et al.* Effect of various extraction methods on the physicochemical properties, antioxidant, and anti-inflammatory activities of mung bean (*Vigna radiata* L.) skin polysaccharides. Int. J. Biol. Macromol. 2025; 143969. https://doi.org/10.1016/j.ijbiomac.2025.143969
16. Baliyan S, *et al.* Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022; 27(4):1326. https://doi.org/10.3390/molecules2704132
17. HDT M. *In vitro* anti-inflammatory egg albumin denaturation assay: An enhanced approach. JONAM. 2023; 7(3):1-6. https://doi.org/10.23880/jonam-16000411
18. Moran M, *et al.* Characterisation of human keratinocyte cell lines for barrier studies. JID Innov 1: 100018. 2021. https://doi.org/10.1016/j.xjidi.2021.100018
19. Iosageanu A, *et al. In vitro* wound-healing potential of phenolic and polysaccharide extracts of Aloe vera gel. J. Funct. Biomater. 2024; 15(9):266. https://doi.org/10.3390/jfb15090266
20. Balko S, *et al.* A robust and standardised approach to quantify wound closure using the scratch assay. Methods and protocols. 2023; 6(5):87. https://doi.org/10.3390/mps6050087

21. Wubante Demilew WD, Adinew G and SA Seyfe Asrade. Evaluation of the wound healing activity of the crude extract of leaves of *Acanthus polystachyus* Delile (*Acanthaceae*). 2018. https://doi.org/10.1155/2018/2047896
22. Belachew TF, *et al.* *In vivo* evaluation of wound healing and anti-inflammatory activity of 80% methanol crude flower extract of *Hagenia abyssinica* (Bruce) JF Gmel in mice. Evidence-Based Complementary and Alternative Medicine. 2020; 2020(1):9645792. https://doi.org/10.1155/2020/9645792
23. Assar DH, *et al*. Wound healing potential of liquorice extract in rat model: Antioxidants, histopathological, immunohistochemical and gene expression evidence. Biomed. Pharmacother. 2021; 143:112151. https://doi.org/10.1016/j.biopha.2021.112151
24. Papin JF, Vahrson W, and Dittmer DP. SYBR green-based real-time quantitative PCR assay for detection of West Nile Virus circumvents false-negative results due to strain variability. J Clin Microbiol. 2004; 42(4):1511-1518. https://doi.org/10.1128/JCM.42.4.1511-1518.2004
25. Badshah SL, *et al.* Isolation, characterisation, and medicinal potential of polysaccharides of *Morchella esculenta*. Molecules. 2021; 26(5):1459. https://doi.org/10.3390/molecules26051459
26. Teymoorian SK, Nouri H, and Moghimi H. *In-vivo* and *in-vitro* wound healing and tissue repair effect of Trametes versicolor polysaccharide extract. Scientific Reports. 2024; 14(1):3796. https://doi.org/10.1038/s41598-024-54565-0
27. Zhu K, *et al.* Study of the ability of polysaccharides isolated from *Zizania latifolia* to promote wound healing in mice via *in vitro* screening and *in vivo* evaluation. Food Chemistry. 2025; 464:141810. https://doi.org/10.1016/j.foodchem.2024.141810
28. Al-Hammood O, *et al.* Deferoxamine-loaded trilayer scaffold containing propolis and sulfated polysaccharides promotes *in vivo* wound healing through angiogenesis stimulation. ACS Appl. Mater. Interfaces. 2025; 17(16):23484-23498. https://doi.org/10.1021/acsami.4c20030
29. Petrović JD, *et al.* A mixture containing 5% polysaccharide extract of *cerioporus squamosus* (huds.) quélet, 5% dexpanthenol, and 0.2% hyaluronic acid shows i*n vitro* and *in vivo* wound healing properties. Pharmaceuticals. 2025. 18(3):416. https://doi.org/10.3390/ph18030416

Author Contribution

Conceptualisation, Y.V. and V.S.; methodology, Y.V., T.V. and V.D; investigation/experimental work, Y.V. and R.K.; data curation, Y.V., S.K. K.D; formal analysis, Y.V and V.S.; writing—original draft preparation, Y.V.; writing—review and editing, V.K.D., S.K., and T.V.; supervision, R.K. All authors have read and agreed to the published version of the manuscript.