Research Article
Open Access

Life Sciences, Medicine and Biomedicine

Exploring *Ipomoea pes-caprae* flower extracts as a natural wound healing and antioxidant agent: *In vitro* bioactivity study

Fatihah Nazar[1], Farah Anisah Abdul Razak[1], Dhia Airina Noor Salizam[1], Dhipan Raj Subramaniam[1], Khaizuran Shahiran Mohd Izhan[1], Thirukanthan Chandra Segaran[2] and Suvik Assaw[1,*]

[1]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Mengabang Telipot, Kuala Nerus, Terengganu, Malaysia.*
[2]*Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Mengabang Telipot, Kuala Nerus, Terengganu, Malaysia.*

**Correspondence:* aasuvik@umt.edu.my

Received 30 October 2025; Revised 28 December 2025; Accepted 22 January 2026; Published online 29 March 2026

ABSTRACT

Wound repair can be disrupted by oxidative stress and enzymatic degradation of extracellular matrix (ECM) proteins, and current synthetic treatments are often limited by side effects. While the leaves and stems of *Ipomoea pes-caprae* are known for their bioactivity, the flowers remain largely unexplored for their therapeutic potential. This study aims to evaluate the antioxidant activity, enzyme inhibition, and wound healing potential of methanolic extracts from *I. pes-caprae* flowers (IPFME). Dried flowers were sequentially extracted with hexane, ethyl acetate, and methanol. Extracts were screened for total phenolic content (TPC) and antioxidant capacity (DPPH assay), after which the methanolic extract (IPFME) was further evaluated for anti-elastase and anti-collagenase activity, cytotoxicity (MTT assay), and fibroblast migration (scratch assay). Methanol extraction produced the highest yield (11.6%) and phenolic content (0.38 mg GAE/g ± 0.003) compared to hexane and ethyl acetate extracts. Antioxidant analysis revealed strong radical scavenging activity with IPFME showing an IC_{50} of 0.06 mg/mL close to gallic acid (0.02 mg/mL). In enzyme inhibition assays, IPFME inhibited collagenase activity by 38.5% and elastase activity by over 90% at 1 mg/mL with inhibition levels comparable to EGCG. Cytotoxicity testing on MRC-5 fibroblast cells revealed an IC_{50} of 0.05 mg/mL, establishing safe, non-toxic concentrations for subsequent assays. Scratch assays further demonstrated accelerated wound closure, with IPFME-treated fibroblasts achieving 95% closure within 24 h, comparable to ascorbic acid (AA). These findings highlight *I. pes-caprae* flowers as a promising source of natural antioxidants and enzyme inhibitors with significant wound healing potential. This multifaceted bioactivity — neutralizing free radicals, inhibiting ECM-degrading enzymes, and promoting fibroblast migration positions the flower extract as a strong candidate for natural therapeutics targeting oxidative-stress-impaired wound healing.

Keywords: *Antioxidant; collagen; fibroblast; Ipomoea pes-caprae and wound healing*

INTRODUCTION

As the body's largest organ, the skin plays a key defensive role by shielding internal tissues from external harm, including physical injuries, infections, UV radiation, and harsh environmental conditions (Gushiken et al., 2021; Albahri et al., 2023). Despite its resilience, the skin remains susceptible to damage from trauma, burns, surgery, or disease (Rodrigues et al., 2018). A wound is defined as a break in the continuity of the skin or underlying tissues (Wang et al., 2023). Wounds are often categorized as acute or chronic based on their clinical appearance, and prolonged healing can lead to infection or other complications (Freedman et al., 2023). The process of wound healing is complex and highly coordinated, encompassing overlapping phases of hemostasis, inflammation, proliferation, and tissue remodeling (Wilkinson & Hardman, 2020; Criollo-Mendoza et al., 2023; Sobeh et al., 2023). Efficient healing is crucial to restore tissue integrity and prevent long-term issues such as chronic wounds or delayed recovery (Kolimi et al., 2022).

https://doi.org/10.28916/lsmb.10.1.2026.243

Excessive formation of reactive oxygen species (ROS) leads to oxidative stress, which hinders the normal healing process (Ukaegbu et al., 2025). Under normal physiological conditions, the body maintains a balance between ROS and antioxidant defenses, but factors such as UV exposure, pollution, and poor nutrition can disrupt this equilibrium (Lin et al., 2025). Persistent oxidative stress damages biomolecules like DNA, proteins, and lipids, contributing to skin aging and chronic diseases including diabetes and cardiovascular disorders (Rinnerthaler et al., 2015; Alfadda & Sallam, 2012). In the skin, UV-induced photodamage is a well-recognized example of ROS-mediated injury that delays wound closure (Wei et al., 2024; Lin et al.,2025). Importantly, excessive ROS not only causes direct cellular damage but also promotes inflammatory responses that enhance the activity of extracellular matrix (ECM)-degrading enzymes, including collagenase and elastase, thereby further impairs wound repair (Diller & Tabor, 2022). Collagenase breaks down collagen, a key structural protein, and excessive activity delays healing and weakens tissue (Mathew-Steiner et al., 2021; Xue & Jackson, 2015). Similarly, neutrophil elastase degrades elastin and collagen during inflammation, and its overactivation can hinder repair and lead to scarring, making anti-collagenase and anti-elastase strategies valuable for enhancing wound closure (Kolaczkowska & Kubes, 2013; Sarangthem et al., 2021).

Although synthetic drugs and dressings are available, many are associated with side effects, resistance or limited efficacy (Cedillo-Cortezano et al., 2024). This has encouraged the exploration of natural products as safer alternatives due to their combined antioxidant, anti-inflammatory and ECM-protective effects. In particular, marine and coastal plants have gained interest as functional sources of bioactive metabolites with relevance to wound management (Karma et al., 2024; Gushiken et al., 2021). Among many coastal plants, *Ipomoea pes-caprae* often called beach morning glory or “tapak kuda,” has long been valued in traditional medicine for treating wounds, inflammation, stings, and jellyfish envenomation (Xavier-Santos et al., 2022). These traditional applications are consistent with biological activities associated with antioxidant protection and preservation of skin structural integrity, which are critical for limiting oxidative damage and excessive ECM degradation during wound healing. Earlier studies have shown that its leaves and stems possess potent antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties likely due to a diverse range of secondary metabolites such as phenols, flavonoids, triterpenes, and alkaloids (Akinniyi et al., 2022; Kumar et al., 2015; Nuskiya et al., 2023).

However, research on the flowers’ part remains limited (Tamuddin et al., 2023) even though they contain colorful pigments and phenolic constituents that could support skin repair and regeneration (Akinniyi et al., 2022). The goal of this study is to evaluate antioxidant activity in *I. pes-caprae* flower extracts from various solvents. The most potent extract was further tested for collagenase and elastase inhibition, cytotoxicity, and fibroblast migration effects *in vitro*, using MTT and scratch assays on MRC-5 cells. By focusing on this less explored part of the plant, this study aims to provide new insight into its possible application as a natural treatment for oxidative stress-related skin damage.

MATERIALS AND METHODS

Sample collection

The flower samples of *Ipomoea pes-caprae* were collected along the Universiti Malaysia Terengganu beaches (5˚4147813N, 103˚0874282E) in November 2022. Species identification was made by comparison with standard regional floras and online herbarium resources (MyBIS, 2025; NParks, 2025). In addition, species confirmation was supported by published literature reports (Salim et al., 2020; Akinniyi et al., 2022).

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. Methanol, hexane, and ethyl acetate were obtained from R&M Chemicals (Selangor, Malaysia). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), porcine pancreatic elastase, and Coomassie Brilliant Blue (CBB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco’s Modified Eagle Medium high glucose (DMEM-high glucose), penicillin–streptomycin (Pen-Strep), and MTT reagent were procured from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS) and trypsin were supplied by Gibco, Thermo Fisher Scientific (Waltham, MA, USA). Phosphate-buffered saline (PBS) was purchased from Oxoid (Basingstoke, England), while bacterial collagenase type I was obtained from MedChemExpress (Monmouth Junction, NJ, USA). All other solvents and reagents used were of analytical grade.

Extract preparation

Sequential extraction using hexane, ethyl acetate, and methanol was employed to fractionate compounds based on polarity, with non-polar compounds extracted by hexane, moderately polar compounds by ethyl acetate, and polar compounds by methanol, ensuring a comprehensive recovery of bioactive constituents.

The dried plant samples (100 g) were ground into a fine powder and subjected to maceration in 500 mL of three different organic solvents which are hexane, ethyl acetate, and methanol. The mixtures were placed in an incubator shaker at room temperature (100 rpm) for 24 hours and then filtered. This process was repeated three times with fresh solvent added each time and filtration performed every 24 hours. All filtrates from the same solvent were mixed and

reduced using a rotary evaporator. The concentrated extracts were transferred into vial bottles and dried in an oven at 40 °C until a uniform weight had been established, indicating complete solvent removal and yielding the crude extracts. The following formula was used to determine each extract's yield percentage:

$$Percentage\ yield\ (\%) = \frac{Weight\ of\ dried\ crude\ extract\ (g)}{Weight\ of\ \ dried\ sample\ used\ (g)} \times 100$$

Total Phenolic Content (TPC) determination

As a foundation, with a few minor adjustments, the Folin-Ciocalteu technique as outlined by Kumar et al. (2015) was used to determine the phenolic content of each extract. A 20 µL sample (1 mg/mL) was placed in a 96-well plate and reacted with 100 µL of Folin–Ciocalteu reagent. After five minutes, 80 µL of sodium carbonate solution was added, and the reaction mixture was kept in the dark at room temperature for one hour. Absorbance was then measured at 750 nm with a UV spectrophotometer. Using a gallic acid standard curve (0-0.5 mg/ml), the TPC was expressed as mg of gallic acid equivalent per gram of dry extract (Aryal et al., 2019).

DPPH radical scavenging assay

Ipomoea pes-caprae flower extracts' antioxidant efficacy was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Assaw et al. (2018). Each extract and gallic acid (used as a standard reference compound) was tested at a range of concentrations from 0 to 1 mg/mL, prepared through twofold serial dilution. For each assay, 50 µL of the crude organic extract or gallic acid was mixed with 100 µL of DPPH solution in a 96-well microplate. Following incubation, the reaction mixtures were maintained in the dark at room temperature for 30 minutes to avoid light-induced degradation. Absorbance values were then recorded at 517 nm using a SpectraMax iD3 microplate reader, with DMSO serving as the blank control. The percentage of radical scavenging activity was determined based on absorbance differences between control and treated samples (Adeyemo et al., 2011). Antioxidant activity was represented as $IC_{50,}$ and the percentage of inhibition was measured as follows:

$$\%\ Inhibition\ = \frac{(Abs\ blank\ -\ Abs\ sample)}{Abs\ blank} \times 100$$

Based on the antioxidant screening, the methanolic extract of *I. pes-caprae* flowers (IPFME) was selected for further assays including enzyme inhibition and cell-based evaluations.

Gelatin digestion assay (anti-collagenase)

The ability of IPFME to inhibit collagenase activity was tested using the gelatin digestion technique, with slight modifications to the method described by Santhanam et al. (2022). Collagenase buffer (50 mM Tris-HCl, 10 mM $CaCl_2$, and 0.15 M NaCl; pH 7.8) containing 0.15% porcine gelatin was used to prepare a 2% agarose solution. The mixture was poured into Petri dishes and left to solidify at room temperature. Wells were created in the gel using sterile 200 µL micropipette tips, and each well was loaded with a mixture of bacterial collagenase type I (0.1 mg/mL) and IPFME at concentrations of 1, 0.5, 0.25, 0.13, 0.06, and 0.03mg/mL. Distilled water served as the negative control, while EGCG (1 mg/mL) was used as the positive control. The plates were incubated at room temperature for one hour, followed by overnight incubation to allow complete digestion. After incubation, the plates were stained with Coomassie Brilliant Blue (CBB), and the clear zones formed were measured to determine the degree of enzyme inhibition.

Anti-elastase assay

The inhibitory activity of the methanolic extract against elastase was determined following a modified approach of Santhanam et al. (2022). The assay was carried out in Tris-HCl buffer (0.2 mM, pH 8.0). Porcine pancreatic elastase (E.C. 3.4.21.36) was freshly prepared in sterile water to obtain a stock concentration of 3.33 mg/mL. The substrate, N-succinyl-Ala-Ala-Ala-p-nitroanilide (AAAPVN), was prepared in Tris-HCl buffer at a concentration of 1.6 mM. For the assay, the enzyme solution was preincubated with different concentrations of *Ipomoea pes-caprae* flower methanolic extract (IPFME) at 1, 0.5, 0.25, and 0.13mg/mL at room temperature. The reaction was initiated by adding the substrate solution. Each reaction mixture had a final volume of 250 µL and contained a final substrate concentration of 0.8 mM and 50 µg/mL elastase. Epigallocatechin gallate (EGCG, 1 mg/mL) served as the positive control, while sterile water was used as the negative control. After incubation, absorbance was read at 410 nm using a microplate reader to determine elastase activity, and percentage inhibition was calculated accordingly:

$$Enzyme\ Inhibition\ Activity\ (\%) = \frac{(Abs\ blank\ -\ Abs\ sample)}{Abs\ blank} \times 100$$

Elastase inhibition data were analyzed descriptively and are presented as mean ± SEM (n = 3), without formal statistical comparison.

Cell based assay using MRC-5 fibroblast cell line

Cell line and culture condition

The human lung fibroblast cell line MRC-5 was obtained from the Animal Cell Culture Facility (ISO 17025), Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu. Information on the original supplier and passage number was not available at the time of receipt. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin and were used within a limited number of passages to ensure experimental consistency. Cells were routinely monitored for morphology and contamination prior to experiments.

Cytotoxicity MTT assay

The cytotoxicity of IPFME was evaluated using the MTT assay, with slight modifications from Beheshti et al. (2021). MRC-5 cells (2×10^5 cells/well) were seeded into 96-well plates and incubated for 24 hours at 37 °C in a humidified atmosphere containing 5% CO_2 to allow cell attachment. The medium used was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin. After attachment, cells were treated with IPFME in serial twofold dilutions ranging from 1000 to 0 µg/mL. Cells exposed to 3% hydrogen peroxide served as the positive control for cytotoxicity. Following 72 hours of incubation, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were kept in the dark for four hours. After discarding the medium, 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The plate was gently shaken and incubated for 10 minutes to ensure complete solubilization, and absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was calculated relative to the untreated control:

$$Cell\ Viability\ (\%) = \frac{Abs\ control}{Abs\ treated\ cells} \times 100$$

Wound scratch assay

The wound healing potential of IPFME was assessed using a scratch assay with MRC-5 fibroblast cells, adapted with minor modifications from Panichakul et al. (2022). Briefly, MRC-5 cells were seeded in 96-well plates similar to the MTT assay until confluent monolayers were formed. A uniform scratch was created in each well using a sterile P10 pipette tip (10µL) in consistent direction with each treatment performed quadruplicate. Images were immediately captured (0 h) under an inverted microscope (Olympus IX51). Cells were then treated with 25 µg/mL and 50 µg/mL IPFME to evaluate sub-cytotoxic and IC_{50}-level effects on fibroblast migration, with ascorbic acid (AA) serving as the positive control. Concentrations were selected based on prior cytotoxicity assessment, ensuring both doses maintained acceptable fibroblast viability. Plates were incubated at 37°C in a humidified atmosphere with 5% CO_2. After 24 h of incubation, images were taken again, and wound closure was quantified using ImageJ software. The migration rate was calculated as the percentage of scratch closure according to the formula:

$$Migration\ rate\ (\%) = \frac{(Distance\ within\ scratch\ (0\ h) - \ Distance\ within\ scratch\ (24\ h))}{Distance\ within\ scratch\ (0\ h)} \times 100$$

Statistical analysis

All experimental results are presented as mean ± standard deviation (SD) or mean ± standard error of the mean (SEM), depending on the data type. Statistical analyses were carried out using GraphPad Prism version 10.4.2. Differences between treatment groups were evaluated using one-way analysis of variance (ANOVA), followed by appropriate post hoc comparisons. Statistical significance was considered at $p < 0.05$.

RESULTS

Extraction yield of *I. pes-caprae* flowers

The fresh flowers of *I. pes-caprae* required 29 days to dry under monsoon season conditions and were found to contain approximately 85% moisture (Table 1). After drying and powdering, the flowers were subjected to sequential solvent extraction: hexane (hex), ethyl acetate (EA), and methanol (MeOH). The extraction yields obtained were 5.8%, 5.3% and 11.6% respectively (Table 2). Among the three solvents tested, MeOH yielded the highest amount of extract which was more than twofold that of hex and EA.

Table 1

Moisture content of I. pes-caprae flowers

Wet Weight (g)	Dry weight (g)	Moisture content (%)
1200	178.5	85.1

Note: Values represent a single batch measurement.

Table 2

Extraction yield of I. pes-caprae flower extracts obtained using different solvents

Solvent	Weight of crude extract (g)	Extraction yield (%)
Hexane	5.8	5.8
Ethyl Acetate	5.3	5.3
Methanol	11.6	11.6

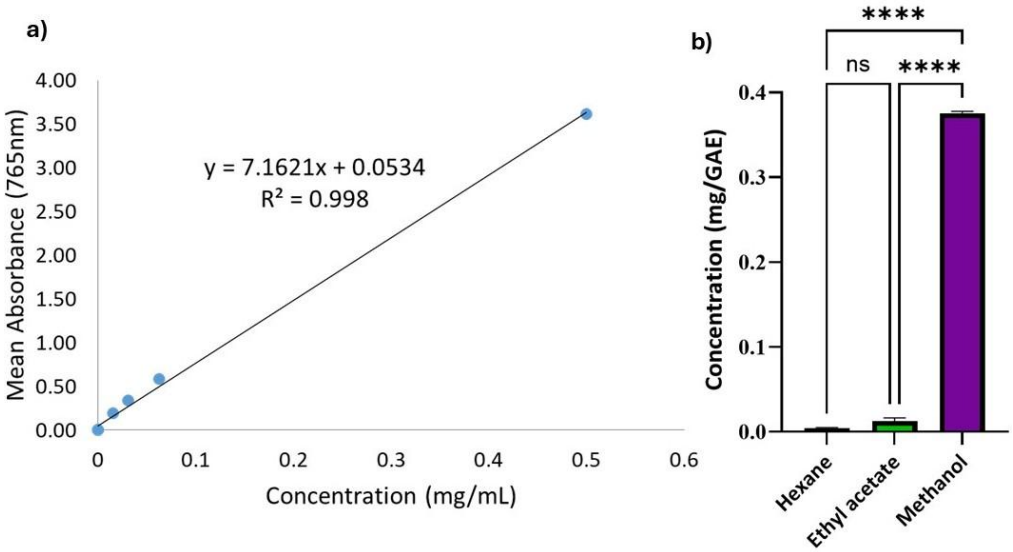
Note: Yields were calculated based on 100 g of dried flower material.

Total Phenolic Content and antioxidant activity (DPPH Assay) of *I. pes-caprae* flower extracts

The TPC of the *I. pes-caprae* flower extracts was determined using a gallic acid standard curve (Figure 1). The methanol extract exhibited the highest phenolic content at 0.38 mg GAE/g ± 0.003 ($p < 0.0001$) compared to ethyl acetate and hexane, respectively (0.01 mg GAE/g ± 0.0 and 0.0 mg GAE/g ± 0.0). Antioxidant activity evaluated using the DPPH assay (Table 3) over a 0–1 mg/mL concentration range, showed that the methanol extract had the lowest IC_{50} at 0.06 mg/mL, followed by the ethyl acetate extract at 0.72 mg/mL. The hexane extract's activity was undetectable within the tested range. The positive control (gallic acid) exhibited an IC_{50} of 0.02 mg/mL (Figure 2). Based on its higher TPC and antioxidant activity, the *I. pes-caprae* flowers' methanolic extract (IPFME) was further evaluated for enzyme inhibitory properties.

Figure 1

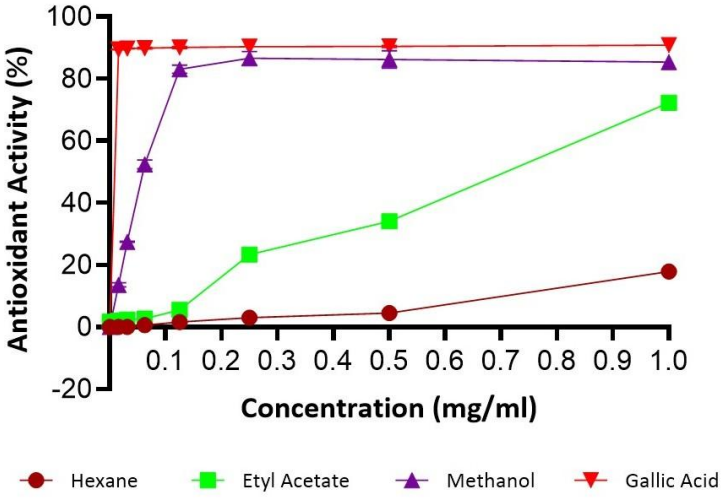
Total Phenolic Content of Ipomoea pes-caprae flower extracts across different solvents using gallic acid standard curve



Note: (a) Standard curve of gallic acid (y = 7.0901x + 0.0835, $R^2 = 0.9987$) used to determine the total phenolic content (TPC) of I. pes-caprae flowers extracted with different solvents. (b) Comparison of TPC in different organic extracts of I. pes-caprae flowers. TPC concentrations were calculated from the gallic acid standard curve. Data are presented as mean ± S.E.M. (n = 3) and analyzed using one-way ANOVA followed by Tukey's post hoc test.

Figure 2

Antioxidant activity of Ipomoea pes-caprae flower extracts



Note: Antioxidant activity of I. pes-caprae flowers extracted with different organic solvents, assessed using the DPPH radical scavenging assay. Data are presented as mean ± S.E.M. (n = 3). Gallic acid was used as the positive control.

Table 3

IC_{50} values of DPPH radical scavenging activity of I. pes-caprae flower extracts

Crude Extract	IC_{50} (mg/ml)
Hexane	> 1.00
Ethyl Acetate	0.72
Methanol	0.06
Gallic Acid (positive control)	0.02

Note: IC_{50} represents the concentration required to scavenge 50% of DPPH radicals.

Collagenase inhibition (gelatin digestion assay)

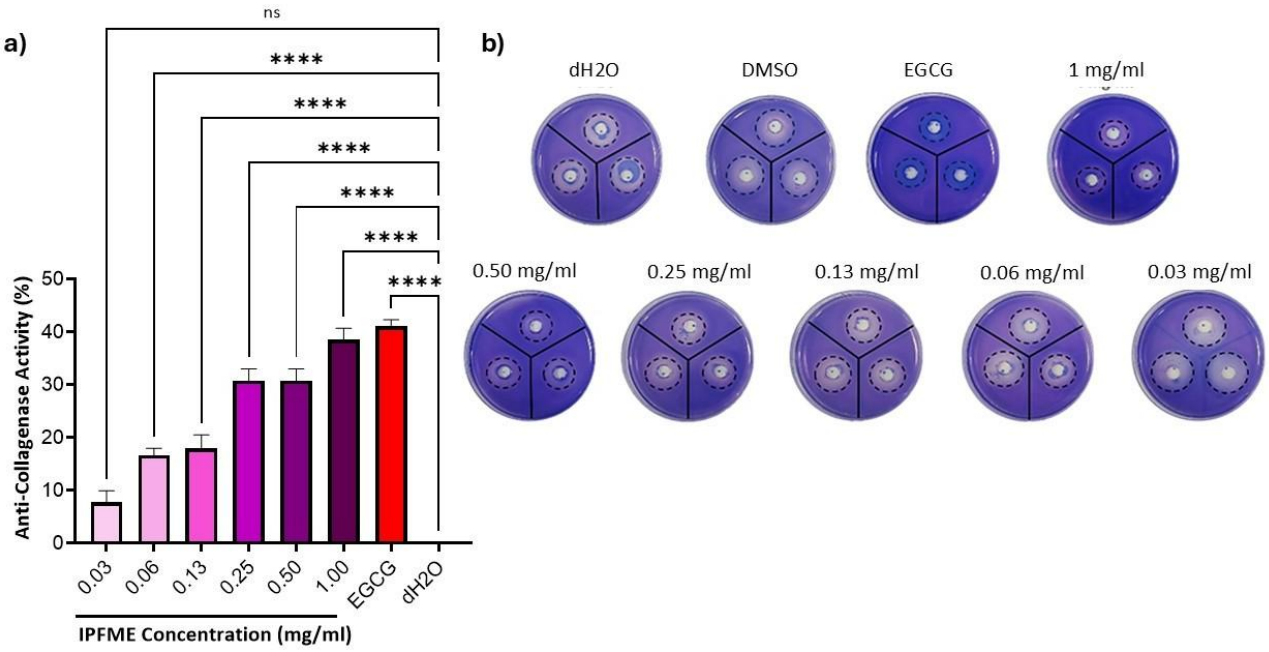
The IPFME exhibited a concentration-dependent inhibitory effect against collagenase activity (Figure 3, Table 4). At the lowest tested concentration (0.03 mg/mL), the inhibition was only 7.7% ± 0.5, but this increased steadily with higher concentrations. At 0.25 and 0.5 mg/mL, inhibition reached 30.8% ± 0.6, while the maximum inhibition observed was at 1 mg/mL (38.5% ± 0.5). This activity was close to that of the positive control, EGCG (41.0% ± 0.3), and was markedly higher than that of the negative control distilled water (dH_2O, 0% ± 0.0). Statistical analysis using one-way ANOVA followed by Dunnett's multiple comparison test showed that IPFME at concentrations ≥0.06 mg/mL produced significantly greater collagenase inhibition compared to the negative control (dH_2O) ($p < 0.0001$), whereas no significant difference was observed at 0.03 mg/mL.

Elastase inhibition assay

The IPFME demonstrated a strong concentration-dependent elastase inhibitory effect (Figure 4, Table 5). Inhibition increased from 21.9% ± 1.5 at 0.13 mg/mL to over 90% at 1 mg/mL. One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test showed that all tested concentrations of IPFME produced a statistically significant inhibition of elastase activity compared with the negative control (dH_2O) ($p < 0.0001$). The negative control (dH_2O) showed no inhibitory activity, confirming that the observed elastase inhibition was attributable to the extract. EGCG, used as the positive control, exhibited strong elastase inhibitory activity, validating the performance of the assay.

Figure 3

Inhibition of collagenase by Ipomoea pes-caprae flower methanolic extract: quantitative analysis and agar plate-based visualization



*Note: Anti-collagenase activity of IPFME. (a) EGCG (positive control) and dH_2O (negative control). Data illustrated as the mean value of the percentage of anti-collagenase activity ± S.E.M (N=3). The data are statistically significant in comparison to the control (dH2O) group with ****p<0.0001. Following Dunnett's multiple comparisons, One-Way ANOVA was implemented to analyse the data. (b) Representative images of collagenase inhibition assay plates showing clear zones of enzyme inhibition at increasing IPFME concentrations compared to controls (EGCG as positive control and DMSO/water as negative control). The intensity and size of the inhibition zones increased with extract concentration indicating dose-dependent inhibitory activity.*

Table 4

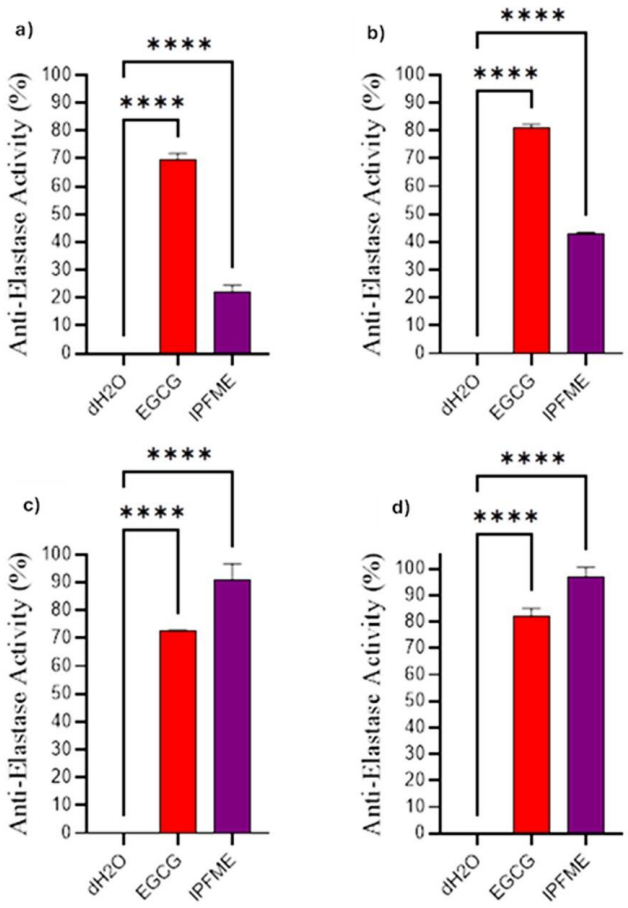
Collagenase inhibitory activity of IPFME

Sample	Concentration (mg/ml)	Anti-collagenase activity (%)
IPFME	0.03	7.7 ± 0.5
	0.06	16.7 ± 0.5
	0.13	17.9 ± 0.3
	0.25	30.8 ± 0.6
	0.50	30.8 ± 0.5
	1.00	38.5 ± 0.5
EGCG (positive control)	1.00	41.0 ± 0.3
Distilled water (negative control)	-	0.0 ± 0.0

Note: Data are expressed as mean ± SEM (n = 3).

Figure 4

Anti-elastase activity by Ipomoea pes-caprae flower methanolic extracts at different concentration



*Notes: Anti-elastase inhibitory activity of IPFME at different concentrations: (a) 0.125 mg/mL, (b) 0.25 mg/mL, (c) 0.5 mg/mL, and (d) 1 mg/mL. Distilled water (dH_2O) was used as the negative control, while EGCG served as the positive control. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test, with comparisons made against the negative control. ****$p < 0.0001$ indicates significant difference compared to dH_2O.*

Table 5

Elastase inhibitory activity of IPFME at different concentrations

Sample	Concentration (mg/ml)	Anti-elastase activity (%)
IPFME	0.13	21.9 ± 1.5
	0.25	42.9 ± 0.3
	0.50	90.8 ± 3.4
	1.00	92.6 ± 4.3
EGCG (positive control)	0.13	69.7 ± 1.2
	0.25	80.8 ± 0.7
	0.50	72.7 ± 0.1
	1.00	81.8 ± 1.8
Distilled water (negative control)	-	0.0 ± 0.0

Note: Data are expressed as mean ± SEM (n = 3).

Cytotoxic effect of IPFME on fibroblasts (MTT assay)

The cytotoxicity of IPFME on fibroblasts was evaluated using the MTT assay. As shown in Table 6 and Figure 5, cell mortality increased progressively with rising concentrations of the extract. At 0.01 mg/mL, mortality was 26.5% ± 0.8, which increased to 76.3% ± 0.7 at 1 mg/mL. The IC_{50} value was calculated as 0.05 mg/mL, indicating moderate cytotoxicity. The positive control, hydrogen peroxide (H_2O_2, 3%), produced 80.1% ± 0.5 mortality, while the vehicle control (DMSO) showed no significant effect (3.9% ± 3.6). These findings suggest that IPFME exerts cytotoxic effects in a concentration-dependent manner, with lower concentrations maintaining acceptable cell viability suitable for subsequent wound healing assays. Based on the IC_{50} value, the concentration of 0.05 mg/ml was selected for subsequent wound-healing evaluation using the scratch assay.

Table 6

Cell mortality of MRC-5 fibroblast cells treated with IPFME at different concentrations

Sample	Concentration (mg/ml)	Cell mortality (%)
IPFME	0.01	26.5 ± 0.8
	0.03	31.7 ± 1.6
	0.06	43.2 ± 3.4
	0.13	61.8 ± 3.9
	0.25	66.2 ± 2.2
	0.50	67.8 ± 3.3
	1.00	76.3 ± 0.7
Hydrogen peroxide (positive control)	3% (v/v)	80.1 ± 0.5
DMSO (vehicle control)	-	3.9 ± 3.6
Untreated control	-	100 ± 0.0

Note: Data are expressed as mean ± S.E.M. (n = 3). DMSO and EGCG were included as vehicle and positive controls, respectively. Untreated cells received culture medium only. The vehicle control contained the solvent without extract, and hydrogen peroxide (3% v/v) was used as a positive control to induce cytotoxicity

Table 7

Cell proliferation rate (%) of fibroblasts measured by scratch assay after treatment with IPFME (25 and 50 µg/mL) for 24 hrs, ascorbic acid (100 mM, positive control), DMSO (0.006%, vehicle control), and untreated cell (negative control)

Treatment	Cell proliferation (%)
Untreated	82.7 ± 2.0
IPFME 25 µg	96.4 ± 2.5
IPFME 50 µg	95.0 ± 1.3
Ascorbic Acid 100Mm (AA)	95.2 ± 1.1
0.006% DMSO	79.8 ± 4.8

Note: Values are expressed as mean ± SEM of triplicate experiments.

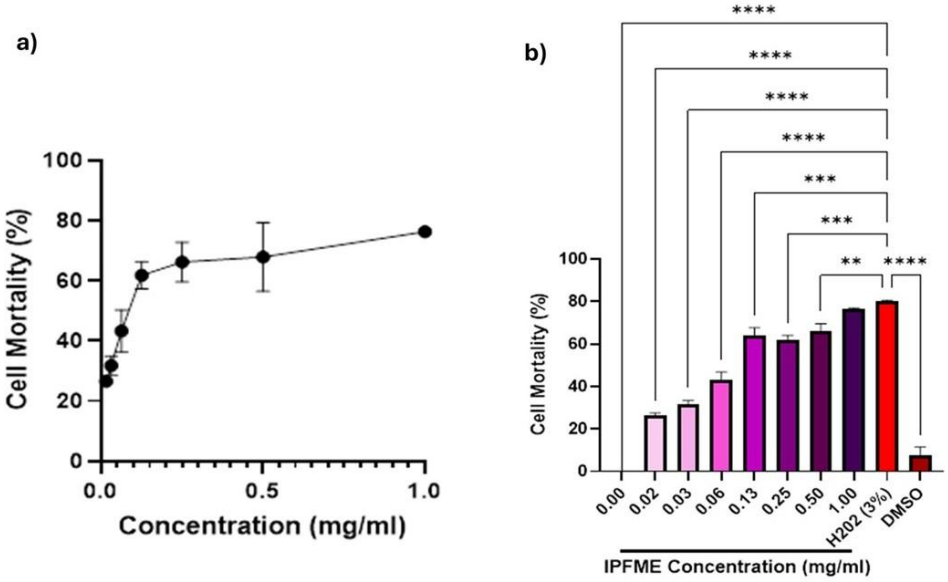
Effect of IPFME on MRC-5 fibroblast cells migration (scratch assay)

In the scratch assay, results in Table 7 showed that fibroblasts treated with 50 µg/mL of IPFME for 24 hours exhibited a significant increase in proliferation (95.05% ± 1.3) compared to the untreated control cells (82.73% ± 2.1) ($p < 0.01$). Treatment with 25 µg/mL (0.025 mg/mL, half IC_{50}) resulted in a slightly higher proliferation rate of 96.4% ± 2.5. No significant difference ($p > 0.05$) was observed between IPFME treatment and ascorbic acid (95.24% ± 1.2), suggesting that the flower extract exerts a wound-healing effect comparable to the positive control. After 48 hours, almost

complete wound closure was observed in all treatment groups, except the untreated control and vehicle control, which still showed a visible gap.

Figure 5

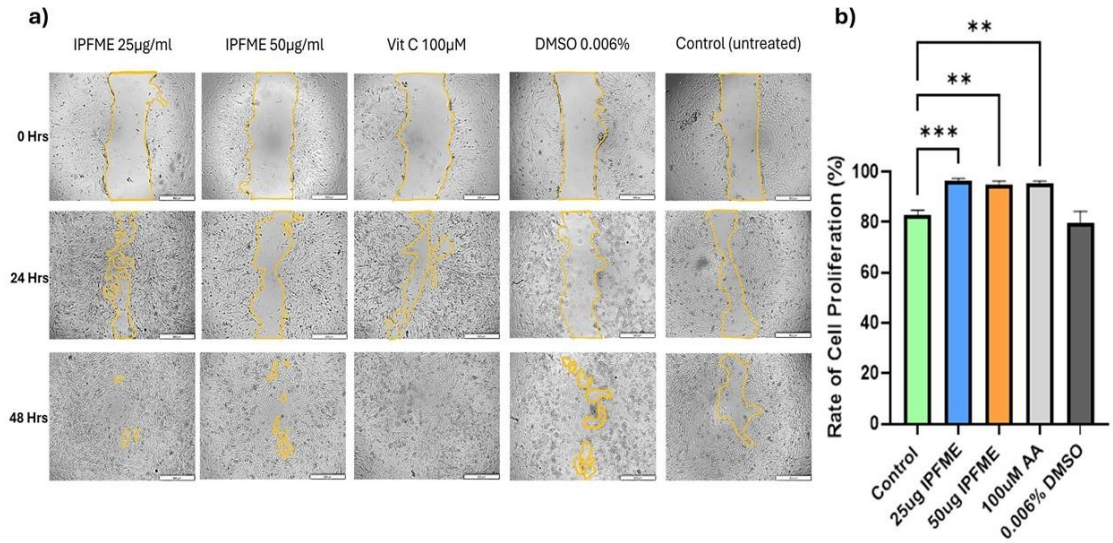
Cytotoxic profile of Ipomoea pes-caprae flower methanolic Extract in MRC-5 fibroblasts: dose–response and IC_{50} determination



*Note: Cytotoxic effect of IPFME on MRC-5 fibroblasts cell determined by the MTT assay. (a) Dose–response curve showing the percentage of cell mortality after treatment with different concentrations of IPFME. The IC_{50} value was calculated to be 0.05 mg/ml. (b) Bar graph representing the percentage of cell mortality at each concentration compared with the vehicle control (DMSO) and positive control (H_2O_2, 1 mM). Data are expressed as mean ± S.E.M. (n = 3). Following Dunnett's multiple comparisons, One-Way ANOVA was implemented to analyse all data. Statistical significance is indicated as **p < 0.01, ***p < 0.001, ****p < 0.0001, ns = not significant.*

Figure 6

In vitro scratch assay analysis: Enhancement of fibroblast migration and wound closure by Ipomoea pes-caprae flower methanolic extract



*Note: Scratch assay showing fibroblast proliferation and migration. (a) Representative images of scratch closure at 0, 24, and 48 hours under inverted microscopy (scale bar = 500 µm, 4× magnification) after treatment with IPFME (25 and 50 µg/mL), ascorbic acid (AA) (100 mM), DMSO (0.006%), and untreated control. (b) Quantitative analysis of cell proliferation rate expressed as percentage of wound closure for 24hrs. Data are presented as mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test, comparing all treatments with the untreated control (**p < 0.01, ***p < 0.001, ns = not significant).*

DISCUSSION

In this study, solvents of varying polarity (hexane, ethyl acetate, and methanol) were employed, with methanol yielding the highest extract percentage. This observation is in line with previous research on *I. pes-caprae* that also employed sequential solvent extraction and reported methanol as the most efficient solvent for obtaining phenolic-rich extracts (Nuskiya et al., 2023). The superior performance of methanol can be attributed to its high polarity, which enhances its ability to penetrate plant cell walls and dissolve intracellular metabolites effectively (Mehmood et al., 2022). Furthermore, methanol has been shown to extract a broader range of phytochemicals compared to less polar solvents. According to Sheeba et al. (2021), both methanol and ethanol extracts of *I. pes-caprae* flowers contain diverse secondary metabolites, including flavonoids, steroids, tannins, and glycosides. The presence of these compounds likely contributed to the higher extraction mass and more potent bioactivity observed in this fraction. Therefore, the results suggest that methanol is a suitable solvent for isolating phenolic and other polar constituents responsible for the biological properties of *I. pes-caprae* flowers.

All flower extracts were found to contain phenolic compounds, but in varying amounts depending on solvent polarity. Hexane yielded the lowest TPC, while methanol extract contained the highest concentration of phenolics, consistent with earlier research demonstrating the efficiency of methanol in recovering polyphenols and flavonoids from I. pes-caprae flowers (Chan et al., 2016). The higher solubility of phenolic compounds in polar solvents such as methanol likely explains this trend. However, since the Folin–Ciocalteu method broadly estimates total phenolics without differentiating among compound classes, the actual flavonoid content in the methanolic extract may be underestimated. A more targeted quantification method for total flavonoids would provide a clearer picture of the phytochemical profile. The antioxidant potential of each extract followed a similar pattern to TPC results. Methanol extract exhibited the lowest IC_{50} value (0.06 mg/mL), signifying the strongest DPPH radical-scavenging activity, whereas ethyl acetate showed moderate activity (0.72 mg/mL) and hexane had minimal effect. This outcome supports the well-documented relationship between phenolic content and antioxidant capacity as phenolics are known to donate hydrogen atoms or electrons to neutralize free radicals (Chaudhary et al., 2023). Comparable associations between phenolic richness and antioxidant activity have been reported not only for *I. pes-caprae* but also for various medicinal plants (Sheeba et al., 2022; Criollo-Mendoza et al., 2023). These findings affirm that phenolic compounds play a major role in the antioxidant efficiency of *I. pes-caprae* flower extracts.

The methanolic extract of *I. pes-caprae* flowers exhibited potent inhibitory effects on collagenase activity, particularly at higher concentrations where its performance approached that of the positive control, EGCG. This result suggests that the extract contains active compounds capable of preserving collagen structure by suppressing enzymatic degradation. Panichakul et al. (2022) previously linked the anti-collagenase activity of *I. pes-caprae* ethanolic extracts to phenolic compounds such as chlorogenic acid, ferulic acid, and isochlorogenic acids, as well as quinic acid derivatives (Teramachi et al., 2005). Mechanistically, collagenase is a zinc-dependent metalloproteinase, and phenolic acids are known to inhibit its activity by chelating the catalytic zinc ion at the active site, thereby disrupting enzyme function (Nagase et al., 2006; Szewczyk et al., 2021). Similar collagenase-inhibitory mechanisms have been reported for phenolic-rich extracts from other medicinal plants (Andrade et al., 2021; Szewczyk et al., 2021; Ghimeray et al., 2015). Therefore, the methanolic extract may serve as a promising natural source of ECM-protective agents that could aid in maintaining skin integrity and supporting wound healing. In addition to collagenase inhibition, the methanolic extract demonstrated pronounced elastase inhibitory activity, in some cases surpassing EGCG. Since elastase plays a crucial role in degrading elastin and weakening skin elasticity, its inhibition is vital for maintaining skin firmness and resilience (Yin et al., 2024). The phytochemical profile of *I. pes-caprae* supports this bioactivity, as its flowers are known to contain abundant phenolic acids and flavonoids that are commonly associated with elastase inhibition (Jakimiuk et al., 2021; Nur et al., 2023). Previous investigations have identified compounds such as chlorogenic acid, caffeic acid derivatives, and flavonoid aglycones in *I. pes-caprae* flowers and aerial parts (Kumar et al., 2020; Panichakul et al., 2022; Teramachi et al., 2005). These molecules are thought to inhibit elastase through direct enzyme interaction or by reducing oxidative stress that indirectly activates elastase (Andrade et al., 2021; Nur et al., 2023). Flavonoids are known to inhibit elastase through hydrogen bonding and hydrophobic interactions with key amino acid residues at the enzyme's active site, which can obstruct substrate binding or alter enzyme conformation (Jakimiuk et al., 2021; Nur et al., 2023). Notably, the elastase inhibitory activity of the extract was stronger than its collagenase inhibition, suggesting a preferential effect on elastin-degrading pathways. This is therapeutically relevant, as excessive elastase activity during the inflammatory phase of wound healing contributes to elastic fiber breakdown, tissue fragility, and delayed remodeling (Criollo-Mendoza et al., 2023). Therefore, the stronger elastase inhibition observed may play a critical role in preserving elastic fiber integrity and promoting effective skin repair and wound healing (Criollo-Mendoza et al., 2023; Yin et al., 2024).

The methanolic extract displayed a concentration-dependent cytotoxic pattern with an IC_{50} of 0.05 mg/mL, indicating moderate cytotoxicity. Importantly, fibroblast viability remained acceptable at concentrations below the IC_{50}, supporting the suitability of sub-cytotoxic doses for wound healing applications. This trend aligns with previous study on *I. pes-caprae* leaf and aerial extracts, where phenolic and flavonoid compounds demonstrated dose-dependent cytotoxicity but were beneficial to fibroblast function at lower concentrations (Panichakul et al., 2022). Comparable results were reported in other medicinal plants where non-toxic, sub-IC_{50} doses enhanced fibroblast proliferation and

antioxidant defense (Criollo-Mendoza et al., 2023; Andrade et al., 2021). The moderate cytotoxicity observed at higher doses may be attributed, at least in part, to the complex composition of the crude flower extract, including the presence of pollen-derived constituents. Certain plant pollen extracts have shown cytotoxic properties against various cell lines (Kustiawan et al., 2014; Karbon & Alhammer, 2020), however their effects on fibroblast cells remains poorly characterized. This suggests that the observed cytotoxicity may not solely reflect the activity of wound-healing phytochemicals but could also involve non-target components inherent to crude flower material. Future studies should therefore address this by comparing extracts prepared from pollen-reduced flower material with unfiltered samples to clarify whether the observed cytotoxicity originates mainly from pollen or other flower metabolites.

At sub-cytotoxic concentrations, the methanolic extract significantly enhanced fibroblast proliferation and migration. The comparison of 25 µg/mL (0.025 mg/mL, half IC_{50}) and 50 µg/mL (0.05 mg/mL, IC_{50}) indicates that both doses effectively stimulate cell growth, with the lower concentration achieving slightly higher proliferation (96.4% ± 2.5 vs. 95.0% ± 1.3), demonstrating that even minimal doses can promote wound healing without inducing cytotoxicity. Both concentrations were comparable to the positive control, ascorbic acid (95.24% ± 1.2), confirming the extract's efficacy. After 48 hours, the almost complete wound closure in treated groups further highlights its ability to support fibroblast migration and tissue repair, whereas untreated and vehicle controls still showed incomplete closure. The inclusion of a lower concentration (0.025 mg/mL) provided valuable insight into how minimal doses could promote wound healing without inducing cellular stress. Previous reports have shown that *I. pes-caprae* leaf extracts stimulate fibroblast proliferation and collagen synthesis (Panichakul et al., 2022), reinforcing the potential of flower extracts as an alternative bioactive source. Phenolic compounds and flavonoids are often credited with such effects due to their antioxidant and signaling-modulatory roles (Szewczyk et al., 2021; Nur et al., 2023). Collectively, these results suggest that *I. pes-caprae* flower extract demonstrates a favorable balance between cytocompatibility and biological efficacy, supporting its potential use in topical wound-healing formulations.

LIMITATIONS

Despite the promising outcomes, several limitations should be considered. The *in vitro* bioassays employed may not fully reflect *in vivo* wound healing responses, and variations in plant source, harvesting conditions, and extraction procedures could influence the chemical profile and bioactivity of the methanolic extracts. In addition, the optimal therapeutic dosage of the extract has yet to be established and warrants further investigation. A key limitation of this study is the moderate cytotoxicity observed at higher extract concentrations. As crude flower extracts were used, non-target constituents, particularly pollen-derived components may have contributed to the cytotoxic effects. Since pollen was not removed prior to extraction, its potential influence cannot be excluded, which may confound the interpretation of cytotoxicity results. Future studies should therefore assess pollen-reduced or filtered extracts to better differentiate the effects of pollen from other bioactive floral metabolites. Furthermore, while the extracts exhibited strong antioxidant and enzyme inhibitory activities, the underlying molecular mechanisms of their wound healing effects were not fully elucidated. Nevertheless, these findings provide valuable preliminary evidence supporting the wound healing potential of *Ipomoea pes-caprae* flower extracts and offer a foundation for future mechanistic and formulation-focused studies.

CONCLUSION

In conclusion, the methanolic extract of *I. pes-caprae* flowers (IPFME) produced the highest yield and showed a strong association between its rich phenolic content and antioxidant potential, with an IC_{50} of 0.06 mg/mL. The extract demonstrated potent enzyme inhibition, particularly against elastase (>90% inhibition at 1 mg/mL), which was notably stronger than its collagenase inhibition, highlighting its potential to protect the skin's extracellular matrix and support wound healing. Moderate cytotoxicity was observed, but concentrations below the IC_{50} were nontoxic and promoted fibroblast proliferation and wound closure *in vitro*. These findings suggest that IPFME could serve as a promising natural source for wound healing formulations. Future work should focus on isolating the specific active compounds, minimizing pollen-related interference, enhancing the therapeutic index, and verifying efficacy and safety through *in vivo* studies.

AUTHOR CONTRIBUTIONS

Fatihah Nazar conceived and designed the study, carried out the experiments, analyzed the data, and drafted the initial version of the manuscript. Farah Anisah Abdul Razak assisted with study design, supervised parts of data acquisition, contributed to interpretation of results, and critically revised the manuscript. Dhia Airina Noor Salizam performed key laboratory work, contributed to statistical analysis, and contributed to drafting of the manuscript. Dhipan Raj Subramaniam was responsible for extract/sample preparation, optimization of assays, and validation of data. Khaizuran Shahiran Mohd Izhan supported methodology development, performed literature review and figure/table preparation, and reviewed the manuscript. Thirukanthan Candra Segaran advised on the in vitro study and

drug delivery. Suvik Assaw supervised the project, provided conceptual guidance, was involved in funding acquisition, led the final approval of the manuscript, and takes overall accountabilities for all parts of the work

ETHICS APPROVAL

Not applicable.

FUNDING

This project was supported by MOHE (FRGS/1/2025/STG01/ UMT/02/2).

CONFLICT OF INTEREST

The authors declare no conflicts of interest in this work.

ACKNOWLEDGEMENTS

The authors would like to express their sincere appreciation to the Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu (UMT) for providing access to the Animal Cell Culture Laboratory. Special thanks are also extended to the Centre of Research and Field Services (CRAFS), UMT for the use of the extraction laboratory facilities. The authors gratefully acknowledge the Faculty of Science and Marine Environment (FSSM), UMT particularly the biodiversity, instrumentation, and marine biotechnology laboratories for their technical support and assistance throughout this research.

REFERENCES

Adeyemo, O. A., Osibote, E., Adedugba, A., Bhadmus, O. A., Adeoshun, A. A., & Allison, M. O. (2018). Antioxidant activity, total phenolic contents and functional group identification of leaf extracts among lemongrass (*Cymbopogon citratus*) accessions. *NISEB J*, *18*(2), 83-91. https://api-ir.unilag.edu.ng/server/api/core/bitstreams/7330eb75-6b27-43e6-9859-a7734e5aa10e/content

Andrade, J. M., Domínguez-Martín, E. M., Nicolai, M., Faustino, C., Rodrigues, L. M., & Rijo, P. (2021). Screening the dermatological potential of plectranthus species components: Antioxidant and inhibitory capacities over elastase, collagenase and tyrosinase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *36*(1), 258-270. https://pubmed.ncbi.nlm.nih.gov/33322969/

Akinniyi, G., Lee, J., Kim, H., Lee, J. G., & Yang, I. (2022). A medicinal halophyte *Ipomoea pes-caprae* (Linn.) R. Br.: A review of its botany, traditional uses, phytochemistry, and bioactivity. *Marine Drugs*, *20*(5), 329. https://doi.org/10.3390/md20050329

Albahri, G., Badran, A., Hijazi, A., Daou, A., Baydoun, E., Nasser, M., & Merah, O. (2023). The therapeutic wound healing bioactivities of various medicinal plants. *Life*, *13*(2), 317. https://doi.org/10.3390/life13020317

Alfadda, A. A., & Sallam, R. M. (2012). Reactive oxygen species in health and disease. *BioMed Research International*, *2012*(1), 936486. https://pubmed.ncbi.nlm.nih.gov/22927725/

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, *8*(4), 96. https://doi.org/10.3390/plants8040096

Assaw, S., Rosli, N. L., Azmi, N. A. M., Mazlan, N. W., & Ismail, N. (2018). Antioxidant and antibacterial activities of polysaccharides and methanolic crude extracts of local edible red seaweed *Gracilaria* sp. *Malaysian Applied Biology*, *47*(4). http://www.mabjournal.com/images/47_4_October_2018/47_04_15.pdf

Beheshti, F., Shabani, A. A., Akbari Eidgahi, M. R., Kookhaei, P., Vazirian, M., & Safavi, M. (2021). Anticancer activity of *Ipomoea purpurea* leaves extracts in monolayer and three-dimensional cell culture. *Evidence-Based Complementary and Alternative Medicine*, *2021*(1), 6666567. https://doi.org/10.1155/2021/6666567

Chan, E. W. C., Baba, S., Chan, H. T., Kainuma, M., & Tangah, J. (2016). Medicinal plants of sandy shores: A short review on *Vitex trifolia* L. and *Ipomoea pes-caprae* (L.) R. Br. *Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)]*, *7*(2), 107-115. http://op.niscpr.res.in/index.php/IJNPR/article/view/12710/

Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Frontiers in chemistry*, *11*, 1158198. https://doi.org/10.3389/fchem.2023.1158198

Cedillo-Cortezano, M., Martinez-Cuevas, L. R., López, J. A. M., Barrera López, I. L., Escutia-Perez, S., & Petricevich, V. L. (2024). Use of medicinal plants in the process of wound healing: a literature review. *Pharmaceuticals*, *17*(3), 303. https://doi.org/10.3390/ph17030303

Criollo-Mendoza, M. S., Contreras-Angulo, L. A., Leyva-López, N., Gutiérrez-Grijalva, E. P., Jiménez-Ortega, L. A., & Heredia, J. B. (2023). Wound healing properties of natural products: Mechanisms of action. *Molecules*, *28*(2), 598. https://doi.org/10.3390/molecules28020598

Diller, R. B., & Tabor, A. J. (2022). The role of the extracellular matrix (ECM) in wound healing: A review. *Biomimetics*, *7*(3), 87. https://doi.org/10.3390/biomimetics7030087

Freedman, B.R., Hwang, C., Talbot, S.G., Hibler, B., Matoori, S., & Mooney, D.J. (2023). Breakthrough treatments for accelerated wound healing. *Science Advances, 9*. https://doi.org/10.1126/sciadv.ade7007

Ghimeray, A. K., Jung, U. S., Lee, H. Y., Kim, Y. H., Ryu, E. K., & Chang, M. S. (2015). *In vitro* antioxidant, collagenase inhibition, and *in vivo* anti-wrinkle effects of combined formulation containing *Punica granatum, Ginkgo biloba, Ficus carica,* and *Morus alba* fruits extract. *Clinical, cosmetic and investigational dermatology*, 389-396. https://doi.org/10.2147/CCID.S80906

Gushiken, L. F. S., Beserra, F. P., Bastos, J. K., Jackson, C. J., & Pellizzon, C. H. (2021). Cutaneous wound healing: An update from physiopathology to current therapies. *Life*, *11*(7), 665. https://doi.org/10.3390/life11070665

Jakimiuk, K., Gesek, J., Atanasov, A. G., & Tomczyk, M. (2021). Flavonoids as inhibitors of human neutrophil elastase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *36*(1), 1016-1028. https://doi.org/10.1080/14756366.2021.1927006

Karbon, M. H., & Alhammer, A. H. (2020). Cytotoxic effect of aqueous-ethanol extract of Typha Domingensis Pers.(pollen) against human breast cancer cells *in vitro*. *Systematic Reviews in Pharmacy*, *11*(10), 1158-1161. https://www.sysrevpharm.org/articles/cytotoxic-effect-of-aqueousethanol-extract-of-typha-domingensis-pers-pollen-against-human-breast-cancer-cells-in-vitro.pdf

Karma, N. I., Mellou, F., Pavlou, P., Siamidi, A., & Varvaresou, A. (2024). Compounds of marine origin with possible applications as healing agents. *Marine Drugs*, *23*(1), 5. https://doi.org/10.3390/md23010005

Kolaczkowska, E., & Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. *Nature Reviews. Immunology*, *13*(3), 159–175. https://doi.org/10.1038/nri3399

Kolimi, P., Narala, S., Nyavanandi, D., Youssef, A. a. A., & Dudhipala, N. (2022). Innovative treatment strategies to accelerate wound healing: Trajectory and recent advancements. *Cells*, *11*(15), 2439. https://doi.org/10.3390/cells11152439

Kumar, A., Paul, S., Kumari, P., Somasundaram, S. T., & Kathiresan, K. (2015). Antioxidant and free radical scavenging activities of *Ipomoea pes-caprae* (L.) R. Br. Extracts. *International Journal of Current Pharmaceutical Review and Research*, 5(4), 91-109. https://impactfactor.org/PDF/IJCPR/5/IJCPR,Vol5,Issue4,Article3.pdf

Kumar, S. S., Badhmapriya, D., & Poornimadevi, C. (2020). A comparative study of bioactive compounds in raw and extract forms of *Ipomoea pes-caprae* (L.) R. Br.(leaf) detected by FT-IR spectroscopy. *Int J Multidisc Res Develop*, *7*(5), 48-52. https://www.allsubjectjournal.com/assets/archives/2020/vol7issue5/7-5-13-665.pdf

Kustiawan, P. M., Puthong, S., Arung, E. T., & Chanchao, C. (2014). *In vitro* cytotoxicity of Indonesian stingless bee products against human cancer cell lines. *Asian Pacific journal of tropical biomedicine*, *4*(7), 549-556. https://doi.org/10.12980/APJTB.4.2014APJTB-2013-0039

Lin, W. T., Chen, Y. J., Kuo, H. N., Kumar, S., Abomughaid, M. M., & Senthil Kumar, K. J. (2025). Ultraviolet B-induced oxidative damage in human skin keratinocytes is alleviated by *Pinus morrisonicola* leaf essential oil through activation of the Nrf2-dependent antioxidant defense system. *Redox Report*, *30*(1), 2527427. https://doi.org/10.1080/13510002.2025.2527427

Mathew-Steiner, S. S., Roy, S., & Sen, C. K. (2021). Collagen in wound healing. *Bioengineering*, *8*(5), 63. https://doi.org/10.3390/bioengineering8050063

Mehmood, A., Javid, S., Khan, M. F., Ahmad, K. S., & Mustafa, A. (2022). *In vitro* total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chemistry*, *16*(1), 64. https://doi.org/10.1186/s13065-022-00858-2

MyBIS (2025). Malaysia Biodiversity Information System. Ministry of Natural Resources and Environmental Sustainability, Malaysia Biodiversity Centre & Forest Research Institute Malaysia. https://www.mybis.gov.my/sp/39708

Nagase, H., Visse, R., & Murphy, G. (2006). Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular research*, *69*(3), 562-573. https://doi.org/10.1016/j.cardiores.2005.12.002

National Parks Board (NParks). (2025). *Ipomoea pes-caprae.* Flora of Singapore. https://www.nparks.gov.sg/florafaunaweb/flora/1/4/1431

Nur, S., Setiawan, H., Hanafi, M., & Elya, B. (2023). Phytochemical composition, antioxidant, in vitro and in silico studies of active compounds of *Curculigo latifolia* extracts as promising elastase inhibitor. *Saudi Journal of Biological Sciences*, *30*(8), 103716. https://doi.org/10.1016/j.sjbs.2023.103716

Nuskiya, A., Sibero, M. T., Setyati, W. A., Andriani, C., & Hendryanti, D. N. (2023). Bioprospecting of katang-katang leaves (*Ipomoea pes-caprae*) from Sumba Island, East Nusa Tenggara: Antimicrobial, antioxidant and secondary metabolites content. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1260, No. 1, p. 012054). IOP Publishing. https://doi.org/10.1088/1755-1315/1260/1/012054

Panichakul, T., Ponnikorn, S., Tupchiangmai, W., Haritakun, W., & Srisanga, K. (2022). Skin anti-aging potential of *Ipomoea pes-caprae* ethanolic extracts on promoting cell proliferation and collagen production in human fibroblasts (CCD-986sk Cells). *Pharmaceuticals*, *15*(8), 969. https://doi.org/10.3390/ph15080969

Rinnerthaler, M., Bischof, J., Streubel, M. K., Trost, A., & Richter, K. (2015). Oxidative stress in aging human skin. *Biomolecules*, *5*(2), 545-589. https://doi.org/10.3390/biom5020545

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2018). Wound healing: A cellular perspective. *Physiological reviews*. https://doi.org/10.1152/physrev.00067.2017

Salim, J.M., Lee, G.E., Salam, M.R., Shahimi, S., Pesiu, E., Jani, J.M., Horsali, N.A.I., Shahrudin, R., Nor, S.M.M., Chong, J.L. and Mohamad, F. (2020). A checklist of vascular plants and uses of some species for livelihood-making in Setiu Wetlands, Terengganu, Malaysia. *PhytoKeys*, *160*, 7. https://doi.org/10.3897/phytokeys.160.52946

Santhanam, R., Karunakaran, T., Sowndhararajan, K., Zulkifli, M. F., Govindan Kothandaraman, M., Aravindhan, V., & Wan Ismail, W. I. (2022). Photoprotective potential, cytotoxicity, and uplc-qtof/ms analysis on bioactive solvent fractions of *Moringa concanensis* nimmo bark. *Evidence-Based Complementary and Alternative Medicine*, *2022*(1), 3781189. https://doi.org/10.1155/2022/3781189

Sarangthem, V., Singh, T. D., & Dinda, A. K. (2021). Emerging role of elastin-like polypeptides in regenerative medicine. *Advances in Wound Care*, *10*(5), 257–269. https://doi.org/10.1089/wound.2019.1085

Sheeba, S. N., Ariharan, V. N., Mary, J. V. J., Bai, S. M. M., & Paul, J. V. (2021). Phytochemical and biological screening of organic solvent extracts of *Ipomoea pes-caprae* flower. *Annals of the Romanian Society for Cell Biology*, *25*(3), 7800-7821. http://annalsofrscb.ro/index.php/journal/article/view/2323/1943

Sobeh, M., Mahmoud, M.F., Monti, D.M., Criollo-Mendoza, M.S., Contreras-Angulo, L.A., pez, N.L., rrez-Grijalva, E.P., nez-Ortega, L.A., & Heredia, J.B. (2023). Wound healing properties of natural products: Mechanisms of action. *Molecules, 28*. https://doi.org/10.3390/molecules28020598

Szewczyk, K., Pietrzak, W., Klimek, K., Miazga-Karska, M., Firlej, A., Flisiński, M., & Grzywa-Celińska, A. (2021). Flavonoid and phenolic acids content and in vitro study of the potential anti-aging properties of *Eutrema japonicum* (Miq.) Koidz cultivated in Wasabi Farm Poland. *International Journal of Molecular Sciences*, *22*(12), 6219. https://doi.org/10.3390/ijms22126219

Tamuddin, Y., Utami, H., Husna, S., Lestari, M., Salawali, R., Towolioe, S., Aras, N., Trifany, A., Papriani, N. (2023). Identification of anticancer and antioxidant potentials of katang-katang flower extract (*Ipomea pes-caprae* Linn) with water solvent. *Jurnal Akta Kimia Indonesia (Indonesia Chimica Acta)*, 38-43. https://journal.unhas.ac.id/index.php/ica/article/view/25353/10495

Teramachi, F., Koyano, T., Kowithayakorn, T., Hayashi, M., Komiyama, K., & Ishibashi, M. (2005). Collagenase inhibitory quinic acid esters from *Ipomoea pes-caprae*. *Journal of Natural Products*, *68*(5), 794-796. https://pubs.acs.org/doi/10.1021/np0500631

Ukaegbu, K., Allen, E., & Svoboda, K. K. (2025). Reactive oxygen species and antioxidants in wound healing: mechanisms and therapeutic potential. *International Wound Journal*, *22*(5), e70330. https://onlinelibrary.wiley.com/doi/10.1111/iwj.70330

Wang, Z., Hu, W., Wang, W., Xiao, Y., Chen, Y., & Wang, X. (2023). Antibacterial electrospun nanofibrous materials for wound healing. *Advanced Fiber Materials*, *5*(1), 107-129. https://doi.org/10.1007/s42765-022-00223-x

Wei, M., He, X., Liu, N., & Deng, H. (2024). Role of reactive oxygen species in ultraviolet-induced photodamage of the skin. *Cell division*, *19*(1), 1. https://doi.org/10.1186/s13008-024-00107-z

Wilkinson, H. N., & Hardman, M. J. (2020). Wound healing: Cellular mechanisms and pathological outcomes. *Open biology*, *10*(9), 200223. https://doi.org/10.1098/rsob.200223

Xavier-Santos, J.B., Passos, J.G.R., Gomes, J.A.S., Cruz, J.V.C., Alves, J.S.F., Garcia, V.B., da Silva, R.M., Lopes, N.P., Araujo-Junior, R.F., Zucolotto, S.M. and Silva-Junior, A.A. (2022). Topical gel containing phenolic-rich extract from *Ipomoea pes-capre* leaf (Convolvulaceae) has anti-inflammatory, wound healing, and antiophidic properties. *Biomedicine & Pharmacotherapy*, *149*, 112921. https://doi.org/10.1016/j.biopha.2022.112921

Xue, M., & Jackson, C. J. (2015). Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. *Advances in wound care*, *4*(3), 119-136. https://doi.org/10.1089/wound.2013.0485

Yin, Q., Zhang, H., Huang, T., Liu, B., Negm, S., & El-Kott, A. F. (2024). Anti-collagenase, anti-elastase, anti-urease, and anti-cancer potentials of Isokaempferide as natural compound: In vitro and in silico study. *Journal of Oleo Science*, *73*(2), 187-199. https://doi.org/10.5650/jos.ess23176

Citation:

Nazar, F., Abdul Razak, F. A., Noor Salizam, D. A., Subramaniam, D. R., Mohd Izhan, K. S., Chandra Segaran, T., & Assaw, S. (2026). Exploring *Ipomoea pes-caprae* flower extracts as a natural wound healing and antioxidant agent: *In vitro* bioactivity study. *Life Sciences, Medicine and Biomedicine*, *10*(1). https://doi.org/10.28916/lsmb.10.1.2026.243

Life Sciences, Medicine and Biomedicine
ISSN: 2600-7207