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In Vitro Assessment of Cell Proliferation and Migration Induced by Crude Lychee Seed Extract in Human Gingival Fibroblast Cells (HGF-1)

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Abstract

- **Background:** Human gingival fibroblasts (HGFs) are central regulators of oral wound healing. Oxidative stress impairs fibroblast viability and motility, potentially delaying wound healing. Lychee seeds are rich in antioxidants, such as phenolics and flavonoids. Their antioxidant effect is expected to improve oral wound healing. Therefore, this study evaluated the effect on wound healing, using the cytotoxicity, proliferation, and migration assay of crude lychee seed extract (LSE) on the HGF-1 cell line.
- **Methods:** Crude LSE was prepared by 95% ethanolic extraction, and its phenolic and flavonoid composition was characterized by ultra-high performance liquid chromatography-mass spectrometry. HGF-1 cells were treated with 0.1–100 μg/mL crude LSE. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in accordance with ISO 10993-5, and morphology was examined by phase-contrast microscopy. Cell proliferation was monitored using the MTT assay over 7 days, and cell migration was evaluated by an *in vitro* scratch-wound healing assay at 8, 16, and 24 h. Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD), and statistical significance was set at $p < 0.05$.
- **Results:** Phytochemical profile of the crude LSE showed 45 compounds across 6 chemical classes, including 15 phenolic and flavonoid species. Crude LSE was non-cytotoxic at 0.1–50 μg/mL (viability > 70%), while 100 μg/mL was marginally below the threshold without reaching statistical significance compared with the control group. Low concentrations (1–20 μg/mL) significantly enhanced HGF-1 proliferation on Day 5, and 0.1 μg/mL produced the highest proliferation on Day 7 ($p < 0.001$ vs control), whereas 50 and 100 μg/mL suppressed growth. In the scratch assay, 0.1–20

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µg/mL accelerated gap closure at 8 h, with 5 and 10 µg/mL showing the greatest migration at 24 h, while 50 and 100 µg/mL delayed closure.

- **Conclusions:** Crude LSE is non-cytotoxic to HGFs at concentrations up to 50 µg/mL and exerts concentration-dependent stimulatory effects on fibroblast proliferation and migration, with 0.1–10 µg/mL emerging as the most promising range. Together with its rich phenolic and flavonoid profile, these findings support further investigation of crude LSE and its purified constituents as candidate agents for promoting oral soft tissue healing.

Keywords: Lychee seed; Human gingival fibroblasts; Cell proliferation; Cell migration; Wound healing

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Background

Human gingival fibroblasts (HGFs) are the most abundant cell type within the oral connective tissue and function as key regulators of oral wound healing. During the repair process, HGFs coordinate extracellular matrix (ECM) synthesis, deposition, and remodeling, providing the structural framework required for re-epithelialization and restoration of tissue integrity (1, 2). The HGF-1 cell line, derived from human gingival tissue, is widely used as a representative *in vitro* model to evaluate fibroblast responses to therapeutic agents intended for oral applications, owing to its ability to recapitulate key proliferative and migratory behaviors observed in primary gingival fibroblasts.

Oral wound healing represents a uniquely demanding biological process, occurring within an environment of continuous microbial exposure, mechanical loading, and constant moisture from saliva. Compared with cutaneous repair, oral mucosal healing is characterized by accelerated kinetics, reduced inflammation, and minimal scarring; however, these advantages may be compromised in cases of chronic or recurrent lesions, such as recurrent aphthous ulcers, periodontal wounds, and therapy-associated oral mucositis, which can induce oxidative stress. In such conditions, the fibroblastic function is frequently impaired, leading to delayed re-epithelialization, disorganized ECM deposition, and persistent inflammation.

An essential factor underlying impaired oral wound healing is oxidative stress, which can perpetuate inflammation, induce ECM degradation, impair angiogenesis, and compromise fibroblast viability and motility (1, 2). Recent evidence indicates that antioxidant interventions may alleviate these deleterious effects and promote a more favorable microenvironment for fibroblast proliferation and migration (3, 4). Consequently, naturally derived antioxidant compounds — particularly those rich in polyphenols and flavonoids — have garnered growing interest as candidate therapeutic agents capable of modulating the oxidative balance of the oral wound microenvironment.

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Lychee (*Litchi chinensis* Sonn.) is a tropical fruit of considerable global agricultural and economic significance, cultivated extensively across Asia and increasingly in subtropical regions of South America, Africa, Oceania, and parts of Europe and North America. Although the pulp is widely consumed worldwide, the seeds — representing a substantial fraction of the fruit by weight — are routinely discarded as agricultural byproducts, generating significant biomass waste that contains potentially valuable phytochemicals. Repurposing such byproducts as evidence-based natural medicines aligns with contemporary global priorities in sustainable resource utilization and the development of plant-derived therapeutics. Phytochemical investigations have consistently reported that lychee seed extract (LSE) contains abundant phenolic and flavonoid constituents (5-9) with potent antioxidant and anti-inflammatory properties (10), positioning it as a promising candidate among natural antioxidant agents.

Despite this strong phytochemical foundation, biological evaluations of LSE have been performed predominantly in the context of dermal wound healing, where it has been associated with enhanced collagen-related activity and systemic anti-inflammatory effects (11). To date, the specific biological effects of LSE within the oral microenvironment — particularly its influence on the proliferation and migration of human gingival fibroblasts — remain substantially underexplored. This gap is particularly notable given the distinct phenotypic, microenvironmental, and regenerative characteristics of oral mucosal fibroblasts compared with their dermal counterparts, which suggest that findings from dermal studies cannot be directly extrapolated to oral applications.

The present study aims to examine how crude LSE affects the HGF-1 cell line by identifying non-cytotoxic concentrations and assessing its influence on fibroblast proliferation and migration. Additionally, it seeks to characterize the constituents of crude LSE.

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Methods

Plant material and extraction

Lychee (Litchi chinensis Sonn. cv. Hong Huay) fruits were obtained from an orchard in Mae Chai, Phayao Province, Northern Thailand. Seeds were manually separated, washed with distilled water, dried at 55°C for 72 hours, ground to a fine powder, and stored at −50°C until use. Extraction was performed at the Physiology Laboratory, School of Medical Sciences, University of Phayao, Thailand, by soaking the dried seed powder in 95% ethanol at a 1:5 (w/v) ratio for 12 hours at room temperature in the dark. The mixture was filtered sequentially through muslin cloth and filter paper, and the extraction-filtration cycle was repeated twice. The combined filtrate was concentrated under reduced pressure at 45°C, and the final extract was lyophilized to yield crude LSE, with an extraction yield of 7.74% (w/w).

Extract preparation and concentration range

Crude LSE powder (1 g) was dissolved in 2 mL of dimethyl sulfoxide (DMSO) to prepare a 50% (w/v) stock solution, which was clarified by centrifugation at 9,000 rpm for 3 minutes. Supernatant was serially diluted in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) to yield working concentrations ranging from 0.1 to 100 µg/mL. The highest concentration (100 µg/mL) was sterilized by filtering through a 0.22 µm syringe filter (NEST® Scientific 331011, USA), and all subsequent working dilutions were prepared from this filtered stock.

Phytochemical analysis

Metabolite profiling of crude LSE was performed by ultra-high-performance liquid chromatography–quadrupole time-of-flight tandem mass spectrometry (UHPLC-QTOF-MS/MS) using a Bruker Elute UHPLC system coupled to a Bruker compact QTOF mass spectrometer and a C18-2 column (100 × 2.1 mm, 1.8 µm) at 40°C. Gradient elution was carried out at 0.2 mL/min using water and methanol, each containing 0.1% formic acid. Detection was

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performed in positive electrospray ionization (ESI) mode over m/z 50–1300, with MS/MS fragmentation acquired in data-dependent acquisition mode at 60 eV. Metabolites were tentatively annotated based on accurate mass and fragmentation patterns to support dereplication and preliminary structural characterization of the constituents.

Cell culture

The human gingival fibroblast cell line HGF-1 (American Type Culture Collection (ATCC) CRL-2014™, Lot 70009862) was cultured in DMEM supplemented with 10% FBS and 1% antibiotic–antimycotic solution at 37°C in a humidified incubator with 5% CO_2. Medium was replaced two to three times per week. At 80–90% confluence, cells were passaged using 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA) and centrifuged at 2,000 rpm for 3 minutes. Passage 5 cells were used for all subsequent experiments, and aliquots were cryopreserved in liquid nitrogen for future use.

Cell cytotoxicity assay

The cytotoxicity of crude LSE on HGF-1 cells was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in accordance with ISO 10993-5 guidelines. Cells were seeded at 20,000 cells per well in 96-well plates and allowed to adhere for 24 hours before treatment with LSE at concentrations ranging from 0.1 to 100 µg/mL for an additional 24 hours. Negative controls (Thermanox™ coverslips) and positive controls (organotin-stabilized polyurethane) were included. Following treatment, 50 µL of MTT solution (1 mg/mL) was added to each well, and the plates were incubated for 2 hours. Formazan crystals were dissolved in 100 µL of isopropanol, and the optical density (OD) was measured at 570 nm using a microplate reader (Bio-Tek Epoch, USA). Cell viability was calculated as a percentage relative to the control. Cell viability below 70% of the control was considered cytotoxic. Each concentration was assessed in eight wells and repeated in triplicate.

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Morphological examination was performed by capturing a micrograph before the addition of the MTT solution.

Cell proliferation assay

The effect of crude LSE on HGF-1 proliferation was assessed by MTT assay at Days 1, 3, 5, and 7. Cells were seeded at 2,000 cells/well in 96-well plates and treated with 100 µl of crude LSE at concentrations of 0.1–100 µg/mL or control (DMEM + 10% FBS), with treatment medium replaced every other day. At each time point, 50 µL of MTT solution (1 mg/mL) was added per well, incubated for 2 hours, and formazan crystals were dissolved in 100 µL isopropanol. Absorbance at 570 nm was measured. Each concentration was assessed in eight wells and repeated in triplicate.

***In vitro* wound healing assay**

The migratory capacity of HGF-1 cells in response to crude LSE was evaluated using a scratch wound assay. Cells were seeded at 100,000 cells/well in 24-well plates and cultured in DMEM supplemented with 10% FBS until reaching approximately 80–90% confluence. The medium was then replaced with serum-free DMEM for serum starvation. A linear scratch was created at the center of each well using a 200 µL sterile pipette tip, and detached cells were removed by gently washing with phosphate-buffered saline (PBS). The cells were subsequently treated with crude LSE diluted in serum-free DMEM at various concentrations (0.1–100 µg/mL) and with a control (DMEM + 10% FBS) for 8, 16, and 24 hours (n = 3 wells per concentration at each time point). Following each treatment period, cells were fixed with methanol and stained with 1% Giemsa. Micrographs were captured across five standardized areas per well at 100× magnification using an inverted microscope (Olympus CKX53, Japan), and wound areas were quantified using ImageJ/Fiji® with the Wound Healing Size Tool plugin. The percentage of wound closure was calculated as:

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$$\textbf{Wound closure\%} = \left(\frac{A_{T=0} - A_{T=\Delta t}}{A_{T=0}}\right) \times 100$$

where $A_{T=0}$ is the initial wound area immediately after scratching and $A_{T=\Delta t}$ is the wound area at each subsequent time point (12, 13). The wound assays were performed in the same manner in triplicate.

Statistical analysis

For each experiment, data normality and homogeneity of variances were assessed using the Kolmogorov–Smirnov and Levene's tests, respectively. Multiple comparisons between LSE concentrations and controls were analyzed using one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post hoc test for cell cytotoxicity, cell proliferation, and the *in vitro* wound-healing assay. Statistical significance was set at $p < 0.05$ with a 95% confidence level. Data are expressed as mean ± standard deviation (SD). All analyses were conducted using IBM SPSS Statistics (Version 29.0; IBM Corp., Armonk, NY, USA).

Results

Phytochemical profile of crude LSE

UHPLC-QTOF-MS/MS analysis of crude LSE in positive ESI mode (m/z 50–1,300) detected approximately 900 spectral features, of which 45 compounds were tentatively annotated using accurate mass matching, MS/MS fragmentation patterns, and database dereplication. The identified constituents were distributed across six major chemical classes, with amino acids and derivatives representing the most abundant fraction (46.4%), followed by phenolics and flavonoids (17.8%), nucleobases and nucleosides (12.3%), fatty acids and lipids (9.6%), sugars and polyols (7.7%), and other constituents (6.2%) (Table 1).

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The phenolic and flavonoid fraction comprised 15 annotated compounds. Condensed tannins, simple phenols, and flavanones dominated this fraction (Table 2). Procyanidin A1 ($C_{30}H_{24}O_{12}$) was the most abundant phenolic constituent, accounting for 18.19% of the phenolic fraction and detected at three distinct retention times (5.96, 7.60, and 8.89 min), consistent with conformational isomers of this A-type proanthocyanidin. The simple phenols resorcinol (15.45%) and 1,3,5-benzenetriol/phloroglucinol (12.73%) collectively represented over 28% of the phenolic fraction. The flavanone pinocembrin was identified at two retention times (12.49 and 14.70 min), contributing 11.19% of the phenolic intensity. The dihydrochalcone phloretin represented 6.87% of the fraction. Additional phenolic constituents consisted of flavonoid glycosides such as rutin (3.70%), isoquercitin (1.29%), 3-coumaric acid (2.59%), coumarins isoscopoletin (2.54%), and 7-hydroxycoumarin (2.35%).

Table 1. Class-level distribution of identified constituents

Chemical class	Relative abundance (% of identified intensity)
Amino acids and derivatives	46.41
Phenolics and flavonoids	17.84
Nucleobases and nucleosides	12.25
Fatty acids and lipids	9.56
Sugars and polyols	7.73
Other constituents	6.21
Total	**100.00**

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Table 2. **Phenolic and flavonoid constituents identified in crude LSE**

No.	Compound	Chemical class	Molecular formula	Retention time (min)	% of phenolic fraction
1	Procyanidin A1	Condensed tannin (A-type proanthocyanidin)	$C_{30}H_{24}O_{12}$	5.96, 7.60, 8.89	18.19
2	Resorcinol	Simple phenol	$C_6H_6O_2$	1.61	15.45
3	1,3,5-Benzenetriol (phloroglucinol)	Simple phenol	$C_6H_6O_3$	1.49	12.73
4	Pinocembrin	Flavanone	$C_{15}H_{12}O_4$	12.49, 14.70	11.19
5	Chromone	Flavonoid scaffold	$C_9H_6O_2$	6.73	10.90
6	Phloretin	Dihydrochalcone	$C_{15}H_{14}O_5$	10.27	6.87
7	3,5-Di-tert-butyl-2-hydroxybenzaldehyde	Phenolic aldehyde	$C_{15}H_{22}O_2$	17.09	4.12
8	2,6-Dimethyl-4-hydroxybenzaldehyde	Phenolic aldehyde	$C_9H_{10}O_2$	9.39	3.85
9	Rutin	Flavonoid glycoside	$C_{27}H_{30}O_{16}$	9.75	3.70
10	3-Coumaric acid	Phenolic acid (hydroxycinnamic acid)	$C_9H_8O_3$	8.15	2.59
11	Isoscopoletin	Coumarin	$C_{10}H_8O_4$	10.79	2.54

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12	7-Hydroxycoumarin (umbelliferone)	Coumarin	$C_9H_6O_3$	7.97	2.35
13	(−)-Naringenin	Flavanone	$C_{15}H_{12}O_5$	12.11	2.21
14	Narcissin	Flavonoid glycoside	$C_{28}H_{32}O_{16}$	10.88	2.02
15	Isoquercitin	Flavonoid glycoside	$C_{21}H_{20}O_{12}$	9.73	1.29
				Total	**100.00**

MTT assay: cell cytotoxicity and proliferation

Cell cytotoxicity

The cytotoxic potential of crude LSE on HGF-1 cells was evaluated using the MTT assay. After 24 hours of exposure, all LSE-treated groups within the 0.1–50 µg/mL concentration range exhibited cell viability above 70%, indicating no cytotoxic potential (Figure 1). At the highest concentration (100 µg/mL), the mean cell viability was 69.58%, which was marginally below the 70% threshold. However, there were no statistically significant differences compared with the control group ($p > 0.05$).

These quantitative results were further supported by morphological analysis via phase-contrast microscopy (Figure 2). Cells treated with low to moderate concentrations of LSE (0.1–20 µg/mL) maintained a typical fibroblast-like appearance, characterized by an elongated, spindle-shaped morphology and healthy cell spreading, comparable to the control group. In contrast, at higher concentrations (50 and 100 µg/mL), early morphological changes indicative of cytotoxic stress were observed. These changes included noticeable cell shrinkage, a more contracted morphology, and a reduction in the number of well-defined elongated cells. Collectively, these findings suggest that the cytotoxic effect of crude LSE is

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concentration-dependent, with the extract remaining biocompatible at concentrations up to 50 µg/mL.

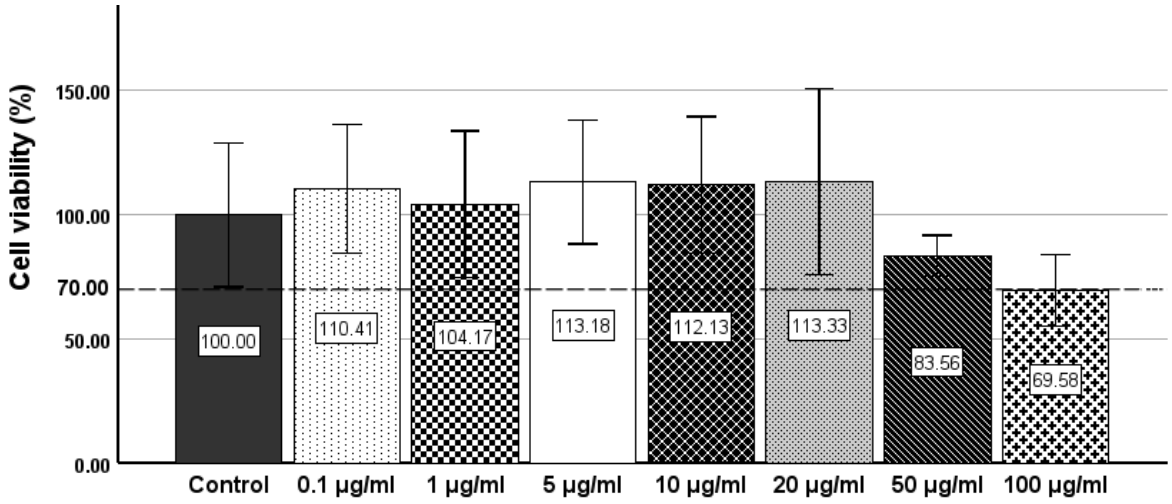


Figure *1***.** Cell viability (%) of HGF-1 cells after treatment with crude LSE at various concentrations (0.1–100 µg/mL). Bars represent the mean, and error bars represent SD (n = *8* per group). The horizontal dashed line at 70% indicates the cytotoxicity threshold. There were no statistically significant differences compared with the control group ($p > 0.05$).

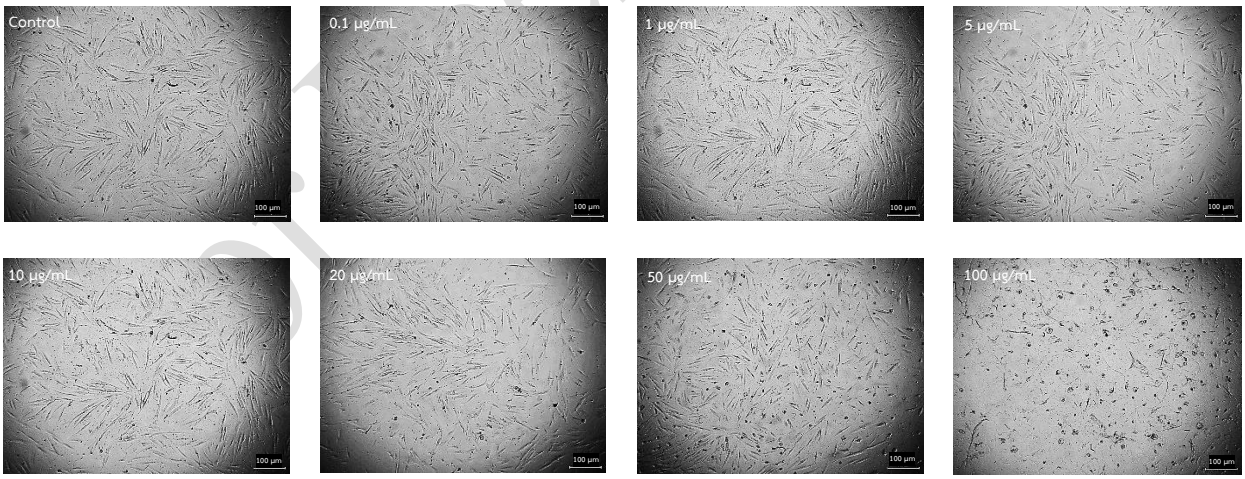


Figure *2.* Morphological observation of LSE-treated HGF-*1* cells in the cytotoxicity assay.

Cell proliferation

The effects of crude LSE on HGF-1 cell proliferation were monitored over 7 days using the MTT assay, with results expressed as OD at 570 nm (Figure 3). At the time points of Day

1 and Day 3, no significant differences in cell proliferation were observed between the crude LSE-treated groups and the control group ($p > 0.05$).

On Day 5, a concentration-dependent stimulatory effect was observed at low concentrations. Cells treated with crude LSE at 1, 5, 10, and 20 μg/mL exhibited significantly higher OD values compared to the control ($p < 0.001$ for 1 and 5 μg/mL, $p = 0.008$ for 10 μg/mL, and $p = 0.039$ for 20 μg/mL). In contrast, the 100 μg/mL group exhibited the lowest proliferative response, significantly lower than those of the 0.1, 1, 5, 10, and 20 μg/mL groups ($p < 0.001$).

On Day 7, the 0.1 μg/mL crude LSE treatment showed the highest overall proliferation, significantly greater than in the control group ($p < 0.001$). Conversely, the 50 μg/mL and 100 μg/mL groups showed a significant reduction in proliferation compared with the control ($p = 0.007$ and $p < 0.001$, respectively). Notably, the 100 μg/mL concentration consistently exhibited a significantly lower proliferative response than all other treatment concentrations on day 7 ($p < 0.001$). These findings indicate that while low concentrations of crude LSE can enhance HGF-1 cell proliferation over time, higher concentrations (50 and 100 μg/mL) significantly suppress cell growth.

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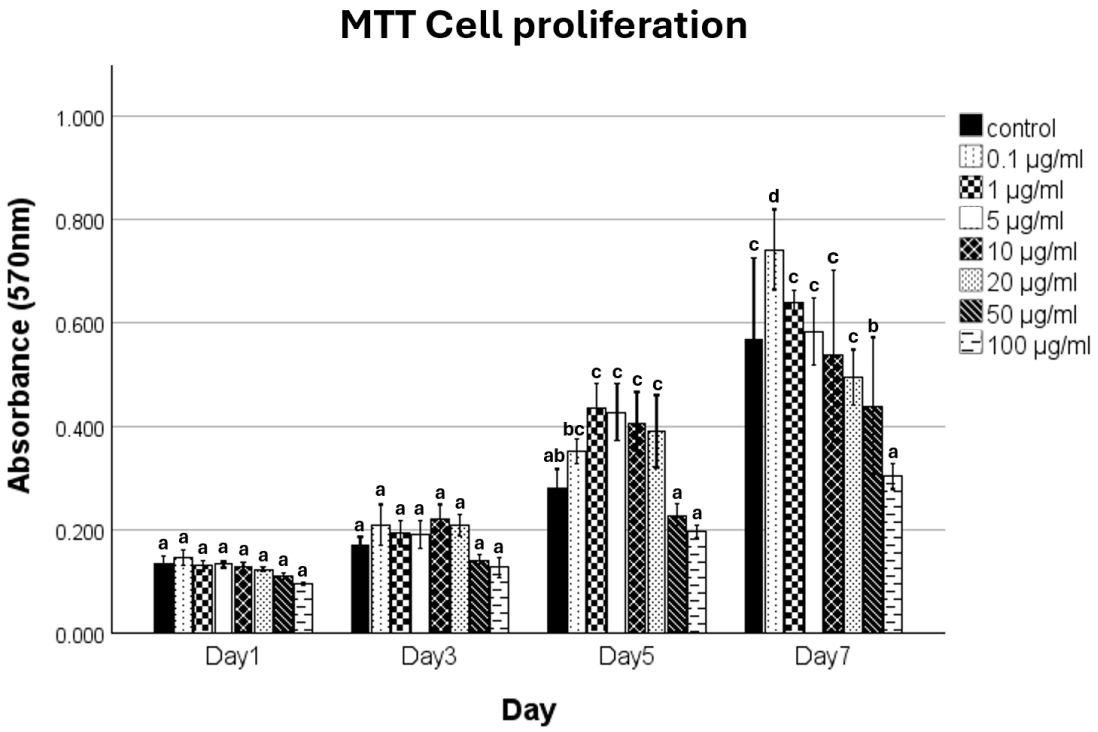


Figure *3***.** Effects of crude LSE on the proliferation of HGF-*1* cells. Bars represent the mean, and error bars represent SD (n = 8 per group each day). On each day, bars sharing at least the same letter are not significantly different ($p > 0.05$).

***In vitro* wound healing assay**

Wound closure increased over time in all groups (Figure 4). At 8 h, low-to-moderate crude LSE concentrations (0.1–20 µg/mL) showed significantly greater gap closure than the control ($p < 0.05$), with 10 µg/mL having the highest gap closure percentage. At 16 h, the migrating areas in the 50 and 100 µg/mL groups were significantly lower than in the control and 0.1–20 µg/mL groups ($p < 0.05$), except that 50 µg/mL did not differ significantly from 1 µg/mL ($p = 0.273$). At 24 h, 5 and 10 µg/mL demonstrated significantly greater migration than the control ($p < 0.001$), whereas 50 and 100 µg/mL remained significantly lower than the other treatment concentrations ($p < 0.05$). Representative micrographs were consistent with these quantitative findings, showing more advanced gap closure in the 5–10 µg/mL groups and delayed closure in the high-concentration groups (50–100 µg/mL) (Figure 5).

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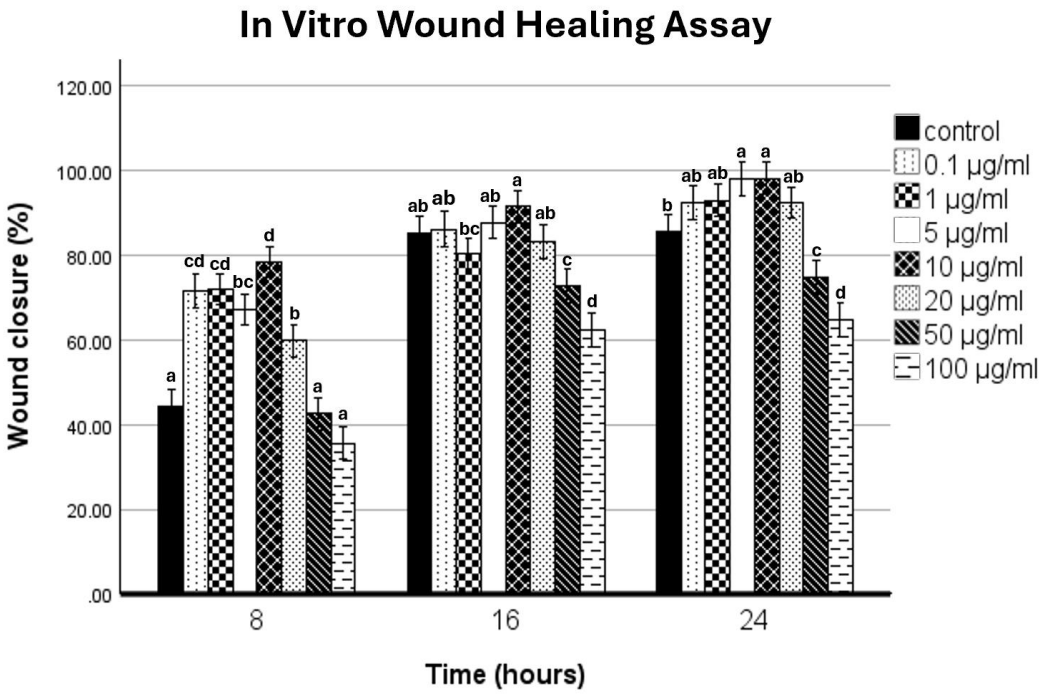
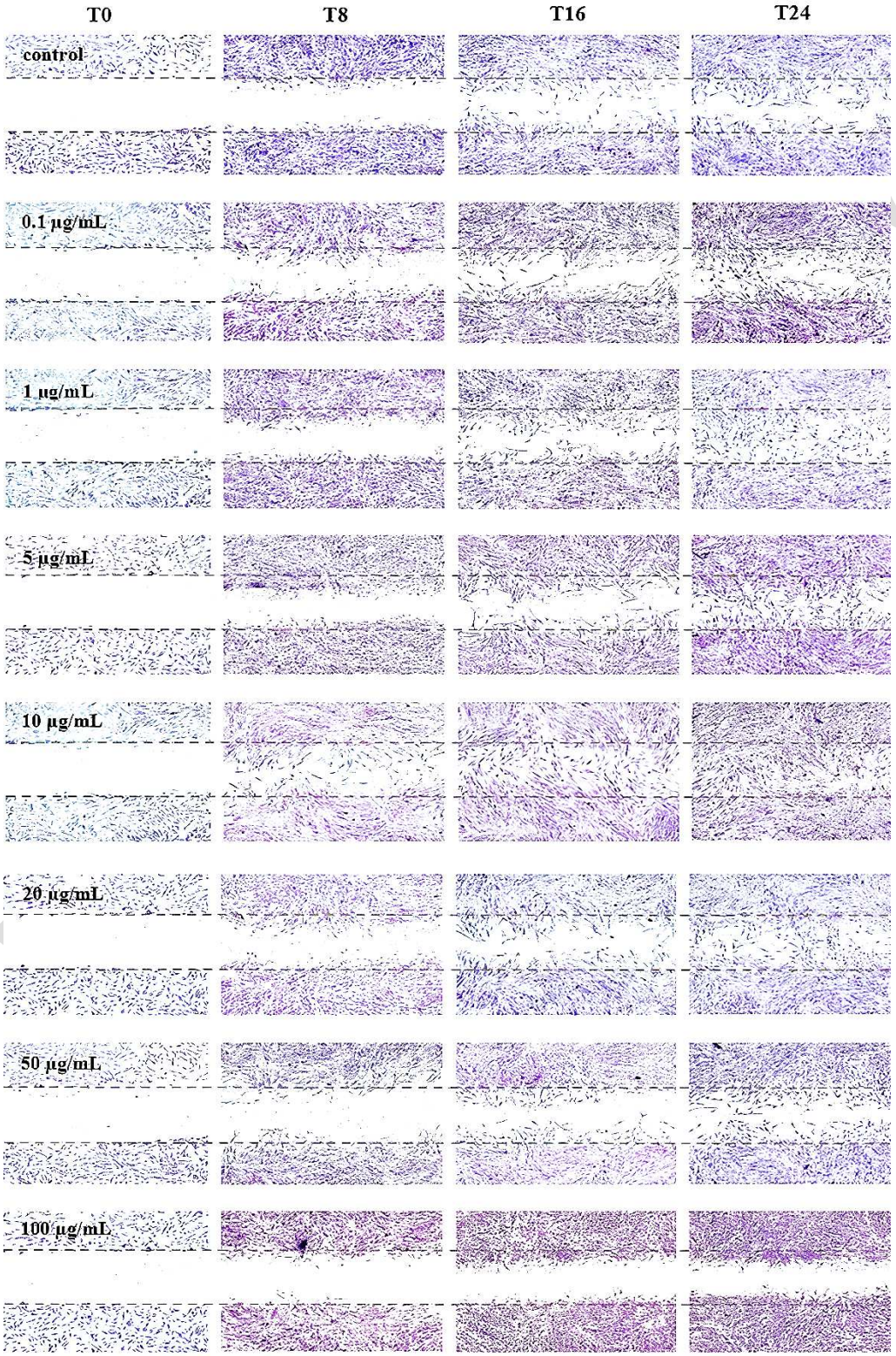


Figure *4***.** Wound closure (%) was quantified at *8*, *16*, and *24* hours after scratch creation. Bars represent the mean, and error bars represent SD (n = 15 per group). Each time, bars sharing at least the same letter are not significantly different ($p > 0.05$).

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Figure *5*. Micrographs of HGF-1 cells treated with crude LSE concentrations (0.1–100 μg/mL) after the wounds were made by scratching the cell culture with a pipette tip. Dotted lines indicate the beginning scratch line at T0.



Discussion

Cytocompatibility screening of crude LSE on HGF-1 cells was performed using the MTT assay, where viability above 70% is considered acceptable for preliminary non-cytotoxicity assessment. The 100 µg/mL group showed mean viability below this threshold, and morphology-based assessment via phase-contrast microscopy revealed reduced cell density and cell shrinkage, consistent with cellular stress, collectively supporting a conservative working range below 100 µg/mL. Although the MTT assay has acknowledged limitations - including potential interference with tetrazolium reduction (14) - it remains widely accepted for screening owing to its reliability, simplicity, and cost-effectiveness (15, 16). Cell migration was evaluated using the *in vitro* scratch assay, a standard technique that quantifies collective cell migration into a reproducible wound gap, approximating certain in vivo wound-closure behaviors and widely adopted for assessing wound-healing effects of natural plant extracts (17-20).

The integration of both endpoints demonstrated a biphasic dose–response relationship: low concentrations (0.1–10 µg/mL) were stimulatory, while high concentrations (50–100 µg/mL) were inhibitory or correlated with cellular stress. The most significant stimulatory effect on proliferation was observed at 0.1 µg/mL, whereas optimal scratch closure was achieved at concentrations of 5–10 µg/mL. A plausible mechanistic explanation suggests that at low concentrations, antioxidant constituents reduce oxidative stress and facilitate fibroblast activity within a more favorable redox environment. Conversely, at higher concentrations, redox imbalance and membrane stress may disrupt cellular homeostasis (21). The discrepancy between optimal concentrations for proliferation and migration is biologically probable, given that these processes are coordinated yet mechanistically distinct and regulated by overlapping yet non-identical pathways. Proliferation is closely associated with cell-cycle–related signaling (22), while migration is more sensitive to cytoskeletal remodeling, adhesion dynamics, and

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ECM interactions — as demonstrated by studies showing that NaCl/KCl conditions augment gingival fibroblast migration via COL1, fibronectin, FAK, and F-actin organization without increasing proliferation (23), and that high glucose suppresses fibroblast migration through inhibition of bFGF–JNK signaling (24). A dose-dependent optimal phenomenon has further been described in which wound closure and ECM gene expression peak within a specific concentration range and decline beyond it (25), consistent with the present findings.

The extraction yield of 7.74% (w/w) obtained under the present conditions (95% ethanol; 1:5 w/v) provided sufficient material for chemical profiling and cell-based assays while maintaining workable solubility for sterile preparation. Although higher solvent volumes and hydroalcoholic concentrations (41–53% ethanol) have been reported to enhance phenolic extractability and antioxidant activity in optimization studies of lychee and longan seeds (26), in our preliminary experience, lower-ethanol extracts tended to be more viscous and to dissolve slowly in DMSO, complicating sterile filtration, accurate dosing, and reproducibility. The selection of 95% ethanol and a 1:5 ratio, therefore, represented a practical balance among recovery, operational simplicity, and downstream solubility requirements. Supportive evidence from a local Thai report also described measurable DPPH radical-scavenging activity in lychee extracts, underscoring that antioxidant potential is preserved across varying solvent polarities (27).

The present findings are consistent with the broader literature demonstrating that polyphenol- and flavonoid-rich plant extracts enhance fibroblast-mediated wound healing at low, non-cytotoxic concentrations. Essential oil combinations at 1–10 μL/mL have been shown to be non-cytotoxic, facilitate scratch closure, and increase collagen deposition (types I/III) in human foreskin fibroblasts (28). Similarly, ethanolic plant extracts characterized by flavonoid and phenolic constituents have been reported to enhance scratch closure without reducing human dermal fibroblast viability over 24–72 hours (29) — a pattern that parallels the present

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findings in HGF-1 cells. More specifically, polyphenolic compounds from Hu Zhang investigated in HGF-1 cells demonstrated significantly enhanced viability at low concentrations (5–25 μg/mL), supporting the interpretation that polyphenol-rich matrices provide a pro-repair microenvironment by balancing oxidative stress and maintaining fibroblast functionality (30).

The phenolic profile of crude LSE presents a mechanistically coherent array of bioactive polyphenols relevant to oral wound healing. Condensed tannins and simple phenols dominate the fraction, with procyanidin A1 (18.19%), resorcinol (15.45%), and phloroglucinol (12.73%) collectively accounting for over 46% of the phenolic and flavonoid fraction. Procyanidin A1 activates the PI3K/AKT signaling axis to restore mitochondrial function and enhance fibroblast migratory capacity (31), while suppressing procollagen type I overproduction in TGF-β1-stimulated fibroblasts (32) - a dual action promoting active tissue repair while preventing pathological scarring. Pinocembrin (11.19%) moderates TGF-β1/Smad signaling to regulate skin fibroblast differentiation (33), and phloretin (6.87%) directly stimulates DNA synthesis and migration in human gingival and periodontal fibroblasts, with specific bioactive compounds at defined concentrations previously shown to enhance oral fibroblast wound-healing responses (34). Rutin and isoquercitin undergo salivary enzyme-mediated hydrolysis to generate free quercetin, which upregulates VEGF and TGF-β1, thereby driving angiogenesis and granulation tissue formation, while suppressing TNF-α, IL-1β, and IL-6 (35, 36). The coumarins isoscopoletin and 7-hydroxycoumarin protect ECM integrity by inhibiting MMP-1 through suppression of p38 MAPK, preventing premature collagen degradation (37, 38).

Supporting in vivo evidence from a 70% ethanolic crude lychee seed extract demonstrated significant dose-dependent anti-inflammatory effects at 150–600 mg/kg in a rat paw edema model, with a reported LD_{50} >5000 mg/kg, indicating a favorable safety margin

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that supports the feasibility of developing topical and local delivery formulations for oral application (39). Mechanistically, lychee seed flavonoids have been proposed to act as Nrf2 activators, with Nrf2/ARE axis activation upregulating antioxidant genes including HO-1, NQO1, SOD, CAT, and GPx, while suppressing NF-κB-related inflammatory pathways (40). This dual antioxidant–anti-inflammatory mechanism is particularly relevant to chronic oral wounds such as recurrent aphthous ulcers and therapy-related oral mucositis, where sustained oxidative stress perpetuates inflammation, promotes ECM degradation, impairs angiogenesis, and delays re-epithelialization (1, 2). Further translational support for the broader proanthocyanidin–flavonoid class - to which lychee seed constituents belong - comes from randomized clinical evidence in patients with recurrent aphthous stomatitis, in which grape seed–derived procyanidins and flavonoids reduced ulcer size, improved wound healing, and provided pain relief (41). Given that lychee seeds similarly contain abundant procyanidins, flavanones, and flavonoid glycosides, these findings collectively reinforce the translational feasibility of crude LSE as a candidate agent for related oral conditions in which inflammation and oxidative stress compromise tissue homeostasis.

Considering some study limitations, biological assays were conducted using a single cell line in a 2D monolayer model, which does not replicate the three-dimensional ECM architecture (42) or the complex immune–vascular microenvironment of oral wound repair (43). The test material comprised a crude extract, and specific active constituents and their mechanisms have not been conclusively identified; observed responses most likely reflect combined multi-compound effects. Despite these limitations, our study is valuable for establishing a baseline of cytocompatibility, identifying a dose-limited optimal range, and prioritizing LSE concentrations for subsequent, higher-complexity models.

Future research should focus on validating active constituents through bioactivity-guided fractionation and confirmatory analytical techniques, including retention-time and UV-

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spectral matching against reference standards (44). Subsequent studies should incorporate 3D *in vitro* systems or an in vivo oral wound model.

Conclusion

Crude LSE is non-cytotoxic to HGFs at concentrations up to 50 µg/mL. Overall, crude LSE exerts concentration-dependent stimulatory effects on HGFs' proliferation and migration. Our results suggest that the LSE of concentrations in the range of 0.1–10 µg/mL appears to be the most promising range for further development as a potential agent to promote oral soft tissue healing.

Abbreviations

ANOVA: analysis of variance; ARE: antioxidant response element; ATCC: American Type Culture Collection; bFGF: basic fibroblast growth factor; CAT: catalase; COL1: collagen type 1; DMEM: Dulbecco's Modified Eagle Medium; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ECM: extracellular matrix; EDTA: ethylenediaminetetraacetic acid; ESI: electrospray ionization; FAK: focal adhesion kinase; FBS: fetal bovine serum; GPx: glutathione peroxidase; HGF-1: human gingival fibroblast cell line 1; HGFs: human gingival fibroblasts; HO-1: heme oxygenase 1; HSD: honestly significant difference; IL: interleukin; ISO: International Organization for Standardization; JNK: c-Jun N-terminal kinase; LD_{50}: median lethal dose; LSE: lychee seed extract; MAPK: mitogen-activated protein kinase; MMP-1: matrix metalloproteinase 1; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB: nuclear factor kappa B; NQO1: NAD(P)H quinone dehydrogenase 1; Nrf2: nuclear factor erythroid 2–related factor 2; OD: optical density; PBS: phosphate-buffered saline; PI3K/AKT: phosphoinositide 3-kinase / protein kinase B; SD: standard deviation; Smad: suppressor of mothers against decapentaplegic; SOD: superoxide dismutase; TGF-β1: transforming growth

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factor beta 1; TNF-α: tumor necrosis factor alpha; UHPLC-QTOF-MS/MS: ultra-high-performance liquid chromatography–quadrupole time-of-flight tandem mass spectrometry; UV: ultraviolet; VEGF: vascular endothelial growth factor.

Declarations

Ethics approval and consent to participate: Not applicable. This study used commercially available established cell lines and did not involve human participants or animals.

Consent for publication: Not applicable.

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