

RESEARCH

Open Access



Wound healing activity of Sweetgum oil (*Liquidambar orientalis* L. balsam): characterization of its mechanism of action on HaCaT human keratinocyte cells and possible responsible active constituents

Muhammed Yusuf^{1*}, Burcu Gökşen-Ekiz¹, Selin Engür-Öztürk², Miriş Dikmen¹, Serkan Levent³ and Yusuf Öztürk⁴

Introduction

Background Sweetgum oil (*Liquidambar orientalis* Mill.; *Styrax liquidus*) is traditionally used in Turkey for wounds and burns, yet its cellular mechanisms remain unclear.

Methods Two locally sourced sweetgum oil samples were tested in human HaCaT keratinocytes. Cytotoxicity was assessed by MTT. Proliferation and migration were quantified using RTCA-DP and scratch assays. COL1A1 mRNA was measured by TaqMan RT-PCR. Phytochemical profiling employed IT-TOF/MS; titrated *Centella asiatica* extract (TECA) was the positive control.

Results Non-toxic concentrations were identified at 0.001–0.1 µg/mL. At 0.001 µg/mL, sweetgum oil significantly increased keratinocyte proliferation and migration in a time-dependent manner (RTCA-DP; $p < 0.05$ to $p < 0.01$), accelerating scratch-wound closure versus control. COL1A1 expression was unchanged. IT-TOF/MS indicated phenylpropyl cinnamate as the dominant constituent in both samples.

Conclusions Sweetgum oil promotes re-epithelialization-related processes primarily by enhancing keratinocyte proliferation and migration rather than collagen induction. These findings support sweetgum oil as a promising natural wound-healing agent and motivate in vivo validation.

Keywords Wound healing, Keratinocytes, Re-epithelialization, Sweetgum oil, Phenylpropyl cinnamate, *Centella asiatica*

*Correspondence:

Muhammed Yusuf
muhammed.yusuf@kocaelisaglik.edu.tr;
mohamed.pharmacia@gmail.com

¹Faculty of Pharmacy, Department of Pharmacology, Kocaeli Health and Technology University, Kocaeli, Turkey

²Department of Pharmacy Services, Tavas Vocational School of Health Services, Pamukkale University, Denizli, Turkey

³Central Research Laboratory (MERLAB), Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

⁴Department of Pharmacology, Faculty of Pharmacy, Istanbul Aydın University, Istanbul, Turkey



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Introduction

Chronic and complicated wounds represent a growing global health and economic burden, with annual treatment costs estimated in the tens of billions of dollars [1]. Healing is often delayed in patients with diabetes, obesity, renal failure, vascular disease, cancer, or advanced age, making wound management a critical challenge. The increasing prevalence of elderly populations further underscores the need for effective and affordable therapies [2–11].

Medicinal plants remain an important source of wound-healing agents, with species such as *Aloe vera*, *Centella asiatica*, *Calendula officinalis*, and *Hypericum perforatum* demonstrating efficacy through experimental and clinical studies. These agents often act by stimulating keratinocyte proliferation, migration, and extracellular matrix remodelling [12–18].

Turkish sweetgum oil (*Styrax liquidus*), obtained from *Liquidambar orientalis* Mill., is endemic to southwest Turkey and has long been used in folk medicine for wounds, burns, and skin irritations [19]. Its composition is dominated by cinnamic acid derivatives, particularly phenylpropyl cinnamate, which may underlie its biological effects [20]. While antimicrobial, antifungal, and anti-inflammatory activities have been reported, no mechanistic studies have examined its wound-healing potential.

This study provides the first experimental evaluation of the wound-healing activity of sweetgum oil in human HaCaT keratinocytes. Using cell-based assays and phytochemical profiling, we investigated its effects on keratinocyte proliferation, migration, and collagen expression, with the aim of identifying active constituents and elucidating possible mechanisms of action.

Materials and methods

Plant material

Sweetgum oil samples obtained from trunk exudate injuries of *Liquidambar orientalis* were purchased from two local producers in the Köyceğiz and Marmaris districts of Muğla, Turkey. Titrated *Centella asiatica* extract (TECA; Bayer, Turkey), known for its established wound-healing activity, was used as the positive control. All samples were stored at 4 °C until analysis.

Stock solutions of SO-1 (Köyceğiz origin), SO-2 (Marmaris origin), and TECA were prepared in DMSO at a concentration of 200 mg/mL. All stock solutions were sterilised using 0.22 µm sterile syringe filters under aseptic conditions. No heat-based sterilisation was applied. Fresh dilutions were prepared in DMEM for each experiment. The final DMSO concentration in all wells did not exceed 0.5% (v/v), and vehicle-only DMSO controls were included.

Cell Culture

The human keratinocyte cell line (HaCaT) was obtained from Cell Line Services (CLS, Germany; passage numbers 25–35 were used). Cells were maintained in DMEM supplemented with 20% fetal bovine serum (FBS), 1% penicillin–streptomycin, 1% L-glutamine, and 1% sodium pyruvate at 37 °C in a humidified incubator with 5% CO₂.

Cells were detached using 2 × trypsin–EDTA, neutralised with complete medium, and centrifuged at 1200 rpm for 5 min. Cell viability (greater than 95%) was confirmed using trypan blue staining before each experiment. Cells were then seeded at 10,000 cells/well in 96-well plates for MTT and RTCA-DP experiments, or 1 × 10⁶ cells/well in 6-well plates for scratch assays.

HaCaT cells were counted before each MTT, RTCA-DP proliferation and migration analysis, scratch assay, and RT-PCR experiment.

MTT Cytotoxicity test

The cytotoxicity of SO-1, SO-2, and TECA was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay [21, 22]. HaCaT cells were seeded into 96-well plates at a density of 10,000 cells/well and incubated for 24 h to allow for attachment.

Stock solutions were diluted in DMEM to obtain the test concentrations. The final concentration of DMSO in all wells was ≤ 0.5% (v/v), and vehicle-only DMSO controls were included.

A two-step screening strategy was used to determine non-cytotoxic concentrations. First, broad concentration ranges (0.01–100 µg/mL; tenfold increments) were tested. In the second step, narrower ranges (0.39–50 µg/mL; twofold increments) were evaluated. Very low concentrations (0.001–0.1 µg/mL) were included to explore potential high-potency effects. After 24 h of treatment, MTT solution was added and incubated for 3 h. The resulting formazan crystals were dissolved in 100 µL DMSO, and absorbance was measured at 540 nm using an ELISA reader. Each condition was tested in eight technical replicates and repeated three times.

Statistical analysis

All experiments were performed in at least three independent biological replicates (n ≥ 3). Data are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 9.0. Differences between groups were evaluated using one-way ANOVA followed by Tukey's post hoc test, and p < 0.05 was considered statistically significant.

Determination of COL1A1 mRNA expression levels by the RT-PCR method

Determination of COL1A1 mRNA expression levels by RT-PCR.

Total RNA was isolated from HaCaT cells treated with SO-1, SO-2, or TECA (0.001, 0.01, or 0.1 µg/mL for 24 and 48 h) using the MagNA Pure Compact RNA isolation system (Roche), and quantified with a NanoDrop 2000 spectrophotometer. RNA concentrations were normalized to 100 ng per reaction.

cDNA synthesis was performed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Germany) with oligo(dT) primers, according to the manufacturer's protocol.

COL1A1 and GAPDH gene expression was quantified using TaqMan probe-based assays (Roche LightCycler® 480 Probes Master). The reaction mix consisted of 10 µL Probe Master, 1 µL primer/probe mix, 5 µL cDNA, and 4 µL nuclease-free water.

PCR amplification was carried out on the LightCycler® 480 system under the following conditions:

95 °C for 10 min (enzyme activation), followed by 45 cycles of 95 °C for 10 s and 60 °C for 30 s.

Gene expression levels were calculated using the $\Delta\Delta C_t$ method, with GAPDH as the housekeeping gene. Each sample was run in triplicate.

Total RNA isolation

After incubation of HaCaT cells with SO-1, SO-2, and TECA at 0.001, 0.01, and 0.1 µg/mL for 24 and 48 h, total RNA was extracted using the MagNA Pure Compact RNA isolation system (Roche). Briefly, cells were collected, centrifuged, washed with 2 mL PBS, and centrifuged again. A volume of 220 µL lysis buffer was added to disrupt the cells, and 200 µL of the lysate was transferred to the instrument. Approximately 50 µL of RNA was isolated within 30 min. The RNA samples were quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Massachusetts, USA) and stored at -20 °C or -80 °C until further analysis.

RNA yield was determined by measuring absorbance at 260 nm and 280 nm. Before cDNA synthesis, RNA concentrations were normalised using NanoDrop results so that exactly 100 ng of RNA from each sample was used.

cDNA synthesis

cDNA was synthesized using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Germany), following the manufacturer's protocol. Oligo(dT) primers were used to target poly(A) RNA. Each reaction consisted of 100 ng of total RNA, 1 µL of Oligo (dT), and nuclease-free water to a total volume of 11.4 µL. Samples were heated at 65 °C for 10 min. Subsequently, 4 µL reaction buffer, 0.5 µL RNase inhibitor, 2 µL dNTP mix, 1 µL DTT, and 1 µL reverse transcriptase were added to reach a final volume of 20 µL. The reaction was incubated at 55 °C for 30 min and at 85 °C for 5 min. The obtained cDNA was used for real-time PCR (Table 1).

Table 1 Reagents added to Lightcycler PCR-compatible clear plates

Order	Chemical	Amount
1	H ₂ O	4 µL
2	Prob Master	10 µL (LightCycler® 480 Probe Master kit REF: 04 707 494 001 for COL1A1)
3	Primer (Taqman)	1 µL (COL1A1/GAPDH)
4	cDNA	5 µL

Determination of mRNA expressions by RT-PCR (Real-time PCR)

The effects of SO-1, SO-2, and TECA at 0.001, 0.01, and 0.1 µg/mL on COL1A1 mRNA expression were measured using TaqMan real-time PCR. The assay was performed using the LightCycler® 480 system (Roche), with COL1A1 as the target gene and GAPDH as the housekeeping gene. Each reaction was performed in triplicate.

Separate wells were prepared for COL1A1 and GAPDH. The plate was placed in the LightCycler® 480, and relative mRNA expression was calculated using the Advanced Relative Quantification module.

The effects of sweetgum oil extracts (SO-1 and SO-2) and TECA were compared to the untreated control.

xCelligence Real-Time Cell Analyse System

The xCELLigence RTCA-DP system was used to evaluate both proliferation and migration of HaCaT cells. This system measures electrical impedance across microelectrodes located at the bottom of the wells, converting the signal into a Cell Index (CI) value, which reflects cell number and behaviour over time.

Proliferation assay:

Background impedance was first recorded by adding 100 µL of DMEM to each well of the E-plates. Then, 100 µL of HaCaT cell suspension (10,000 cells/well) was added. Plates were incubated for 30 min at room temperature to allow for uniform cell attachment, followed by continuous impedance monitoring every 1 h for 24 h. After confirming cell adherence, non-toxic concentrations of sweetgum oil (0.001, 0.01, and 0.1 µg/mL) were added, and impedance was recorded for an additional 48 h. Dose-response curves were generated at 48 h.

Migration assay:

The same RTCA-DP platform was used with CIM-plates for migration analysis. Non-toxic concentrations of SO-1, SO-2, and TECA (0.001–0.1 µg/mL) were applied, and impedance was recorded for 72 h. Migration slope values were calculated from the linear phase of the cell index increase.

Scratch wound healing technique

The migration and wound-closure capacity of HaCaT keratinocytes was evaluated using the scratch assay.

Cells were seeded into 6-well plates at a density of 1×10^6 cells per well and cultured until reaching approximately 85–95% confluence. A linear scratch was gently made in the cell monolayer using a sterile 100 μ L pipette tip. Detached cells and debris were removed by washing with PBS.

Cells were then treated with non-toxic concentrations of sweetgum oil samples (SO-1 and SO-2) and TECA (0.001, 0.01, and 0.1 μ g/mL) in fresh complete medium and incubated for 48 h at 37 °C in 5% CO₂. Three representative images were captured for each well at 0, 24, and 48 h using a Leica DM300 inverted microscope. Wound closure was quantified using the Leica LAS

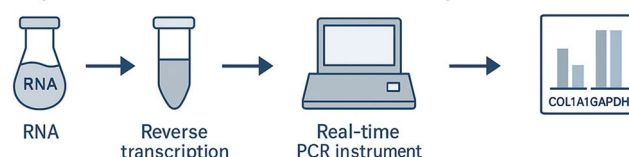
Image Analysis software by measuring the remaining scratch area. Each experiment was performed at least in duplicate.

Chemical analyses of sweetgum oil samples

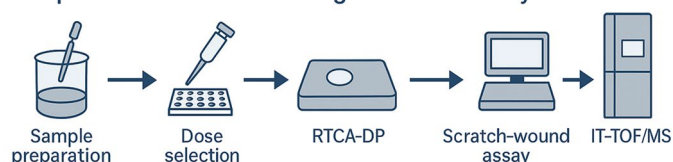
Qualitative and quantitative analyses of the sweetgum oil samples (SO-1 and SO-2) were performed using IT-TOF/MS high-resolution mass spectrometry (Shimadzu, Kyoto, Japan). Samples were diluted in water and methanol, sonicated for 10 min in an ultrasonic bath, and filtered through 0.22 μ m PTFE or CA syringe filters before analysis.

(A)

A TaqMan RT-PCR workflow for COL1A1 quantification



B Experimental workflow diagram of the study



(B)

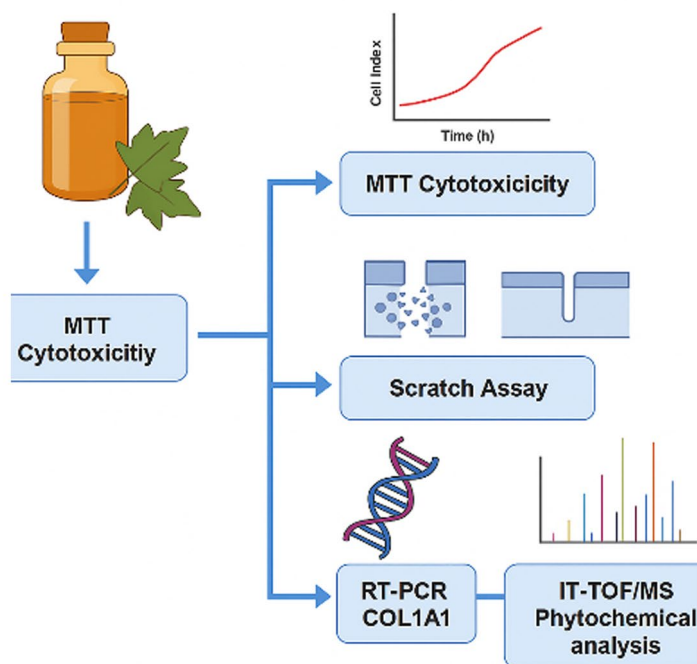


Fig. 1 A Study workflows. A TaqMan RT-PCR workflow for COL1A1 quantification in HaCaT keratinocytes. B Experimental workflow showing dose selection by MTT, real-time proliferation and migration analysis by RTCA-DP, scratch-wound closure assay, and chemical profiling by IT-TOF/MS. B Experimental workflow diagram

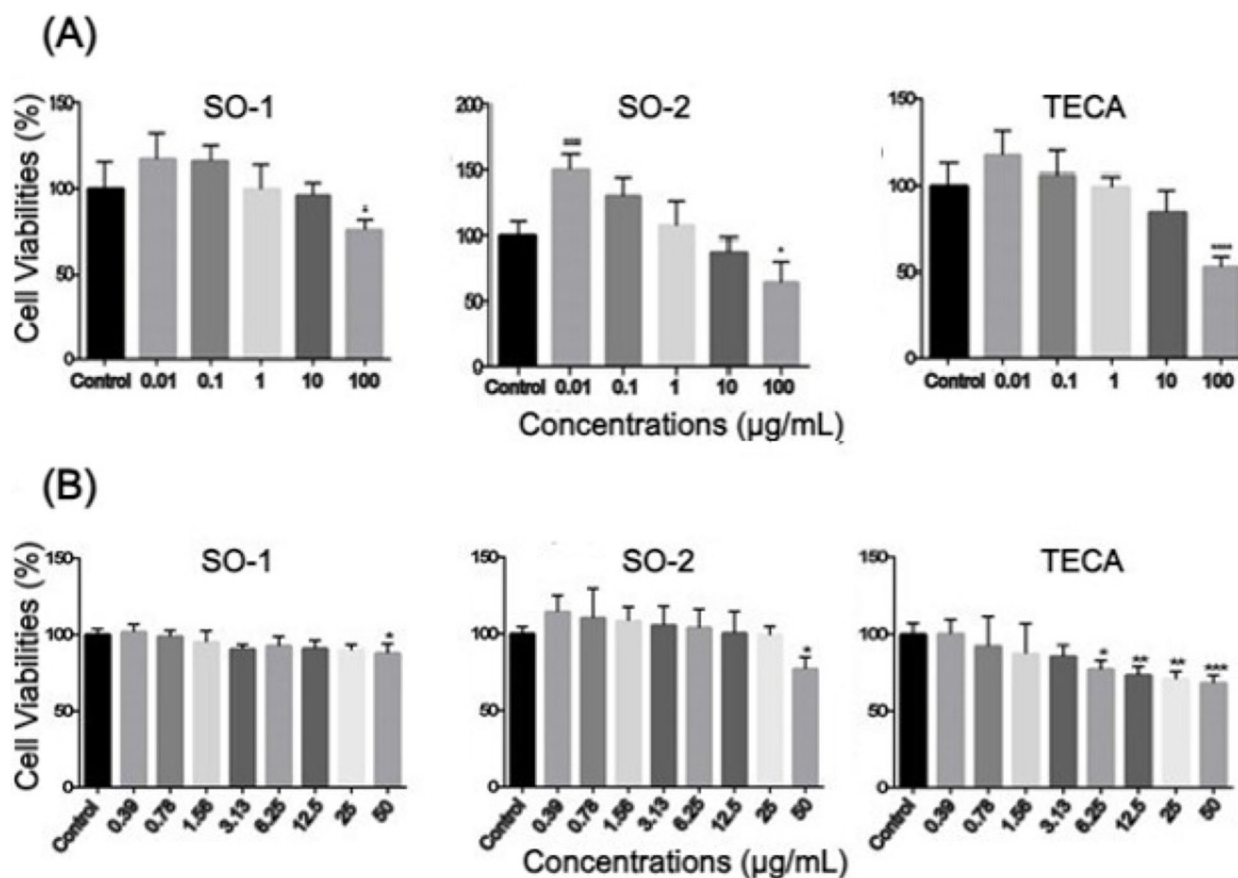


Fig. 2 MTT cell viability result graphs at different concentration ranges: **A** Concentrations of 100, 10, 1, 0.1, 0.01 µg/mL ($n=6$ for each case), **B** Concentrations of 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 µg/mL ($n=6$ for each case; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, statistical significance versus control). Data are mean $\pm$ SD ($n=6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. control

Analyses were conducted using electrospray ionisation (ESI) in both positive and negative ion modes. The mobile phase consisted of a gradient mixture of acetonitrile and water containing 0.1% formic acid. Instrument parameters were as follows: probe voltage ± 3.5 kV (positive/negative ESI), nebulising gas flow 1.5 L/min, CDL temperature 200 °C, heat block temperature 200 °C, and drying gas pressure 200 kPa. The TOF detector voltage was 1.6 kV. The LC unit consisted of dual LC-20AD pumps, a DGU-20A3R degasser, CTO-10ASvp column oven, SIL-20AC autosampler, and SPD-M20A PDA detector.

Chromatographic separation was achieved using a Shim-Pack FC-ODS C18 column (150 $\times$ 2 mm, 2 µm). Data were processed using LCMSsolution software (v. 3.80). Compound identification was performed based on major peak masses using the DrugBank database (drugbank.ca) and literature references. An overview of the experimental design and analytical workflow is shown in Fig. 1.

Results

Determination of non-toxic concentrations in MTT test

In the first step of cytotoxic concentration screening in HaCaT cells, it was determined that 0.001, 0.01, and 0.1 µg/mL concentrations of SO-1 and SO-2 were non-toxic (Fig. 2A). Mild proliferative effects were observed at some concentrations (0.01 and 0.1 µg/mL). The increase

Table 2 For HaCaT cells, RNA Nanodrop results for 48 h

Extracts	Concentrations (µg/mL)	Absorbance
SO-1	0.001	351,6
	0.010	372,6
	0.100	289,3
SO-2	0.001	340,7
	0.010	417,1
	0.100	274,7
TECA	0.001	329,4
	0.010	379,5
	0.100	463
Control		610,4

in proliferation at 0.01 $\mu\text{g/mL}$ for SO-2 was statistically significant ($p < 0.01$), while the effect of SO-1 at the same concentration was minimal and not significant. At 0.1 $\mu\text{g/mL}$, both SO-1 and SO-2 still exhibited proliferative effects, but they were not statistically significant (Fig. 2A).

Similar results were obtained for TECA, which did not show significant proliferative activity at low

concentrations but exhibited strong cytotoxicity at 100 $\mu\text{g/mL}$ ($p < 0.005$) (Fig. 2A).

In the second step of screening, seven concentrations of SO-1 and SO-2 extracts ranging from 0.39 to 25.0 $\mu\text{g/mL}$ were found to be non-toxic. Some concentrations of SO-2 (0.39, 0.78, and 1.56 $\mu\text{g/mL}$) produced slight but statistically insignificant proliferative effects. For both sweetgum oil extracts, cytotoxicity in HaCaT cells was

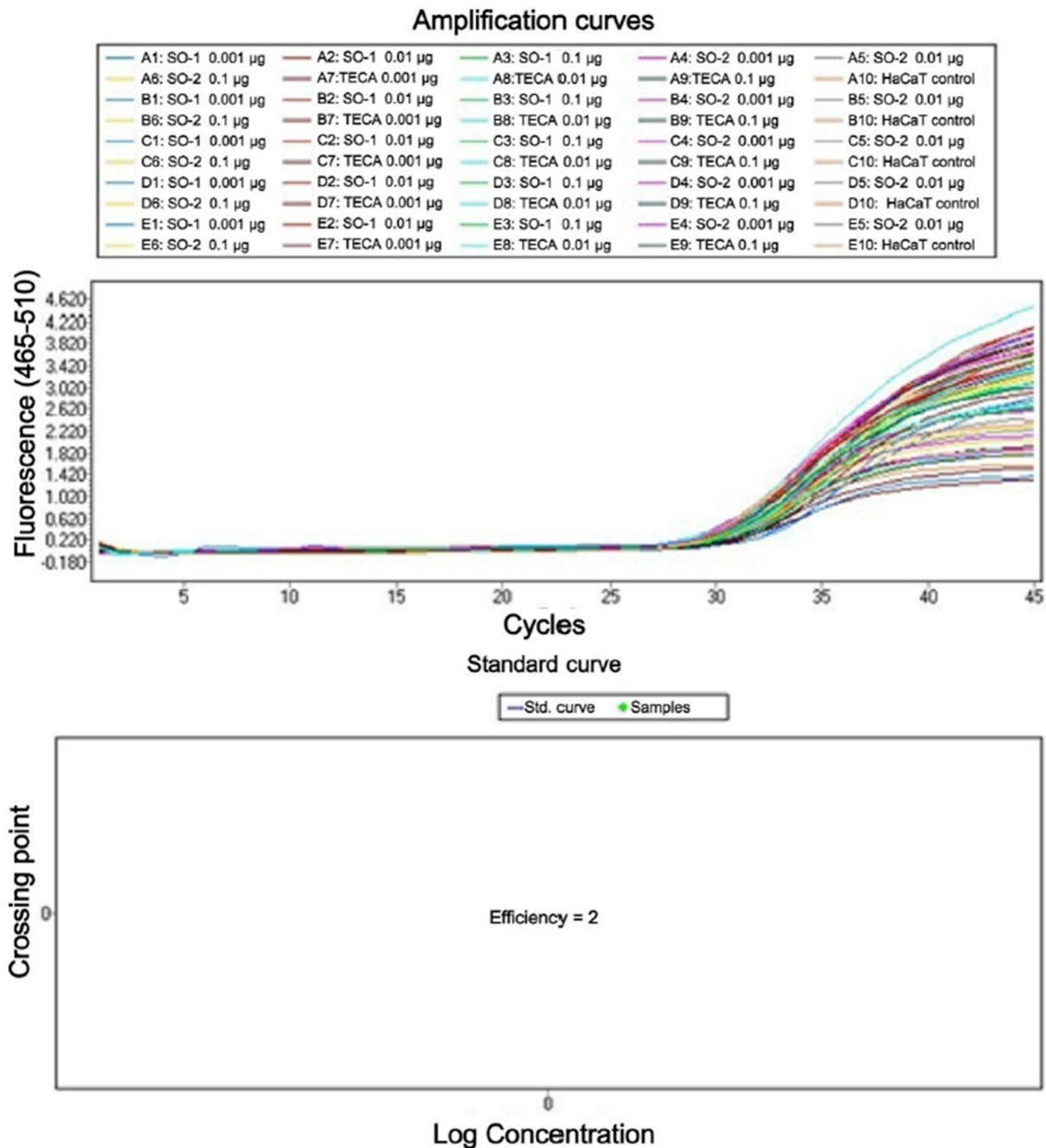


Fig. 3 Amplification curve of COL1A1 gene in HaCaT keratinocyte cells incubated with SO-1, SO-2 and TECA at concentrations of 0.001, 0.01, and 0.1 $\mu\text{g/mL}$ for 24 h. Data are mean $\pm$ SD ($n=6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. control

clearly observed at 50.0 $\mu\text{g/mL}$, where SO-1 and SO-2 induced significant antiproliferative effects ($p < 0.05$). TECA showed a similar profile but demonstrated concentration-dependent cytotoxicity from 6.25 $\mu\text{g/mL}$ onward ($p < 0.05$), becoming highly cytotoxic at 12.50–100.00 $\mu\text{g/mL}$ ($p < 0.01$ – 0.005) (Fig. 2B).

Determination of COL1A1 mRNA expression levels by RT-PCR

Table 2 presents the NanoDrop RNA quantification results for HaCaT keratinocyte cells after 48 h

of treatment. RT-PCR analysis showed that COL1A1 mRNA expression levels were not altered in HaCaT cells treated with SO-1, SO-2, or TECA at 0.001, 0.01, or 0.1 $\mu\text{g/mL}$. Data were analysed using the Software Release LCS480 version 1.5.0.39 (Fig. 3).

Xcelligence real-time cell analyse system

According to RTCA-DP analysis over a 72-h period, sweetgum oil significantly increased HaCaT cell proliferation at the 0.001 $\mu\text{g/mL}$ concentration in a time-dependent manner. The proliferation profiles of SO-1, SO-2,

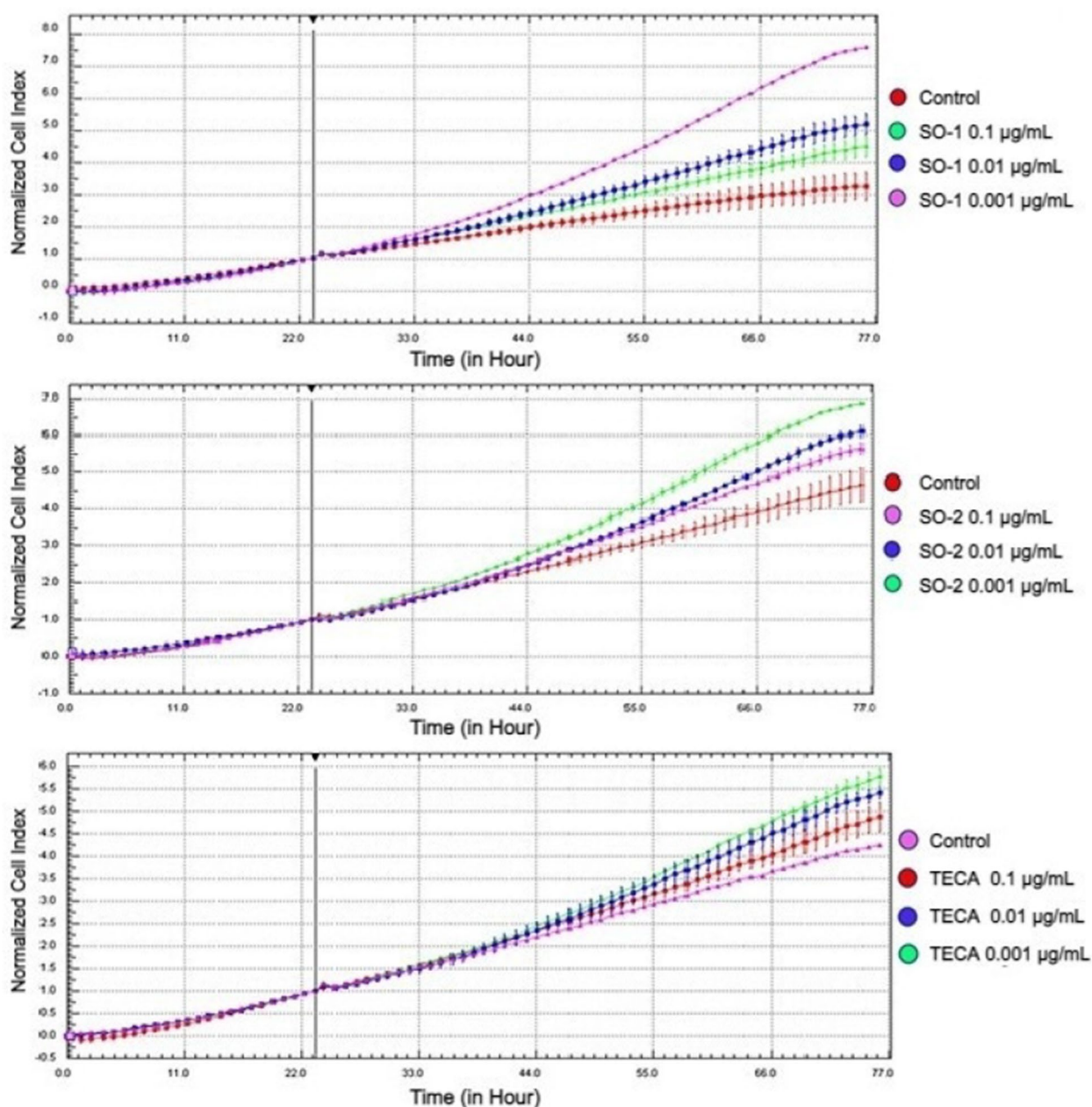


Fig. 4 Results of RTCA-DP cell proliferation assays for SO-1, SO-2 and TECA ($n=6$ for each case) $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. control. Data are mean $\pm$ SD ($n=6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. Control

and TECA are shown in Fig. 4. These results are consistent with the findings obtained from the MTT assay.

Similarly, RTCA-DP migration analysis revealed a significant increase in cell migration at 0.001 $\mu\text{g/mL}$ for all tested samples (SO-1, SO-2, and TECA), again in a time-dependent manner. Migration curves and the corresponding slope histograms are presented in Figs. 5, 6, and 7. Slope values at 0.001 $\mu\text{g/mL}$ were 1.42 ± 0.10 for SO-1 and 1.55 ± 0.12 for SO-2, both significantly higher than the control group (0.88 ± 0.09 , $p < 0.01$).

Scratch wound healing technique

Scratch-wound analysis showed that HaCaT keratinocyte cells treated with non-toxic concentrations of SO-1, SO-2, and TECA exhibited enhanced migration and wound closure within 48 h. Figures 8, 9, and 10 display the wound closure progression at 24 and 48 h following treatment.

At 48 h, wound closure was $68 \pm 5\%$ for SO-1 and $72 \pm 6\%$ for SO-2 at 0.001 $\mu\text{g/mL}$, compared to $40 \pm 4\%$ in the control group ($p < 0.01$). TECA achieved $65 \pm 5\%$ closure under the same conditions. Migration slope values at 0.001 $\mu\text{g/mL}$ were 1.42 ± 0.10 for SO-1 and 1.55 ± 0.12 for SO-2, both significantly higher than control (0.88 ± 0.09 , $p < 0.01$).

Chemical analyses of sweetgum oil samples

Table 3 summarises the IT-TOF/MS high-resolution mass spectrometry results for the sweetgum oil samples (SO-1 and SO-2). The dominant component in both samples was phenylpropyl cinnamate, with relative intensities of 82.1% for SO-1 and 69.5% for SO-2. This was followed by cinnamyl cinnamate at 9.3% and 19.6%, respectively. Other identified constituents included benzyl alcohol, cinnamaldehyde, cinnamic acid, and p-hydroxy cinnamic acid.

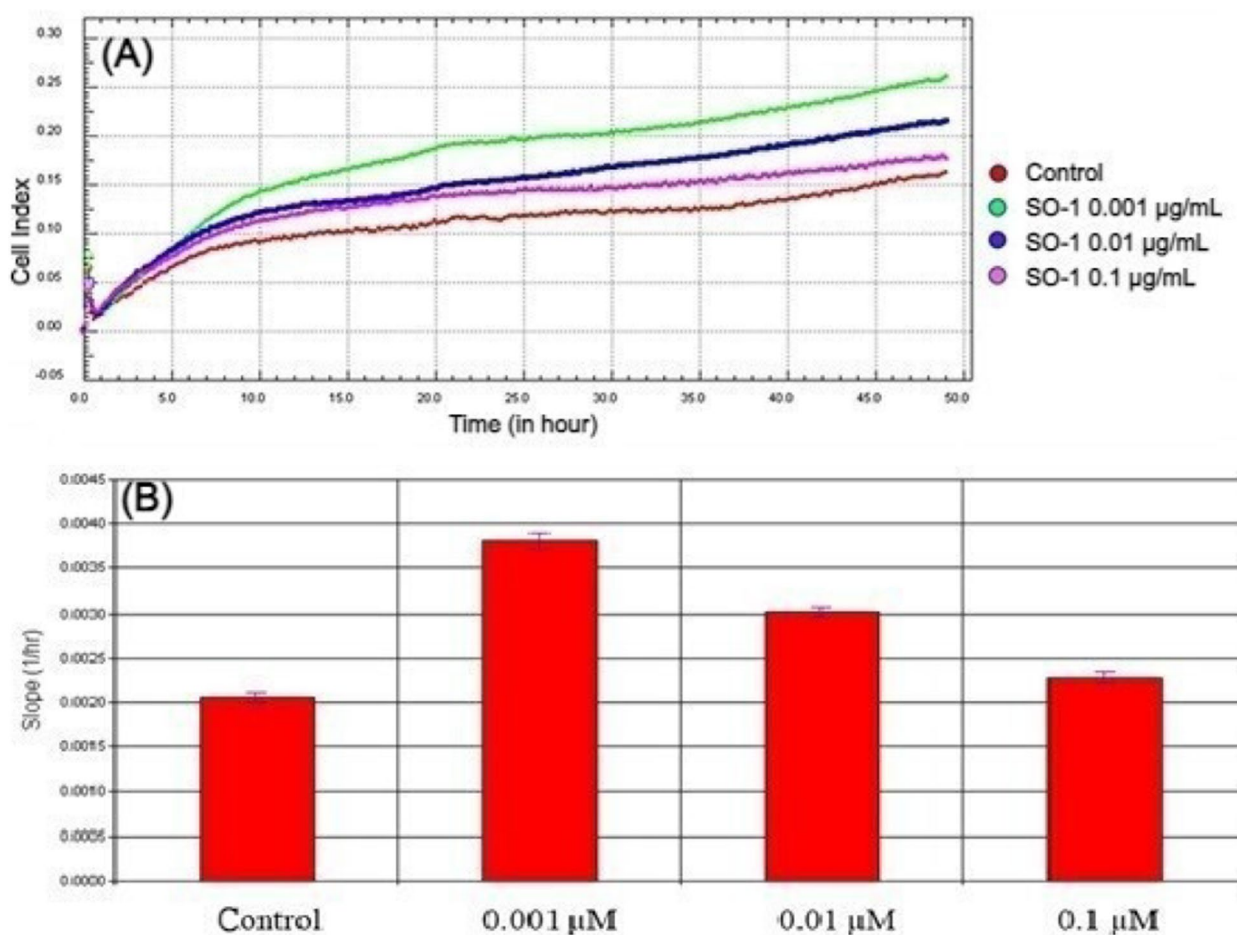


Fig. 5 **A** Graph of time-dependent migration effect of sweetgum oil (SO-1) concentrations on HaCaT keratinocyte cells in RTCA-DP ($n=6$); **B** Slope values of SO-1 concentrations in HaCaT keratinocyte cells in the RTCA-DP system after 72 h ($n=6$; mean $\pm$ standard deviation). Data are mean $\pm$ SD ($n=6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. control

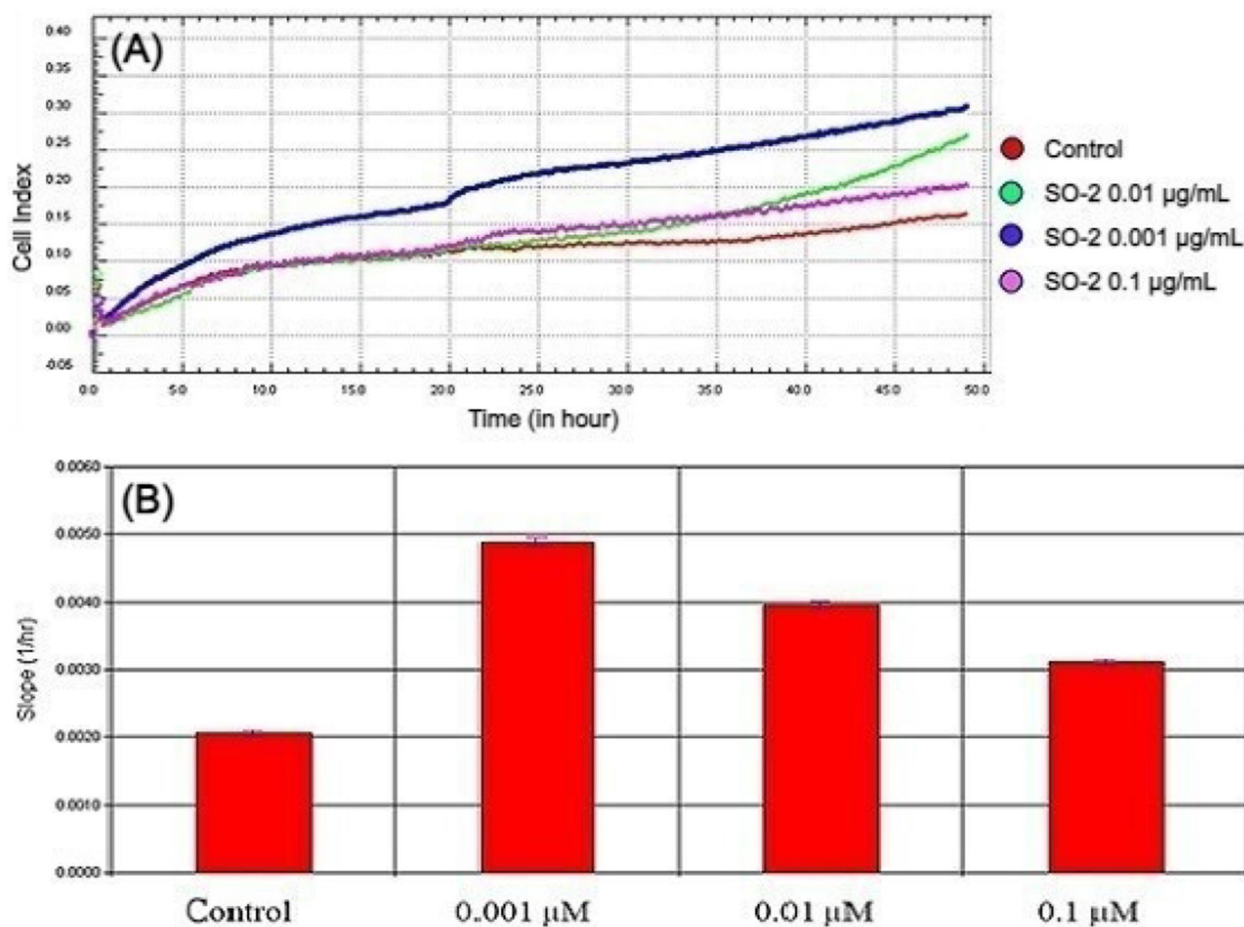


Fig. 6 (A) Graph of time-dependent migration effect of sweetgum oil (SO-2) concentrations on HaCaT keratinocyte cells in RTCA-DP ($n=6$); (B) Slope values of SO-2 concentrations in HaCaT keratinocyte cells in the RTCA-DP system after 72 h ($n=6$; mean $\pm$ standard deviation). Data are mean $\pm$ SD ($n=6$). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. control

Discussion

Sweetgum oil (*Styrax liquidus*) has been traditionally used in Turkey for treating skin lesions, infections, and inflammatory conditions [12]. Previous *in vivo* studies have shown that sweetgum oil accelerates wound repair, primarily through epithelialization and antibacterial activity [23, 24]. Yanik (2016) demonstrated comparable efficacy between sweetgum oil and silver sulfadiazine in burn wounds, reporting improvements in inflammation, collagen accumulation, angiogenesis, and epithelialization [25]. Similarly, Deniz (2010) reported enhanced wound closure when sweetgum oil was combined with collagenase [26]. However, these studies did not investigate the underlying cellular or molecular mechanisms, limiting mechanistic understanding.

Our study is the first to demonstrate that sweetgum oil promotes wound-healing-related processes in human keratinocytes. Using MTT assays, we identified non-toxic concentrations in the range of 0.001–0.1 µg/mL. At 0.001 µg/mL, sweetgum oil significantly enhanced keratinocyte proliferation and migration in both RTCA-DP

and scratch assays ($p<0.05$, $p<0.01$), resulting in accelerated wound closure. Notably, no change was observed in COL1A1 expression, indicating that the effect is not mediated through collagen synthesis. This finding is consistent with the work of Öçsel et al. (2012), who reported no significant effect of sweetgum oil on fibroblast activity or collagen density in a porcine wound model [24]. These data collectively suggest that the mechanism of action differs from agents such as *Centella asiatica*, which enhances wound healing via promotion of collagen synthesis [12]. Instead, sweetgum oil appears to act primarily through stimulation of keratinocyte proliferation and migration, key processes involved in re-epithelialization.

Phytochemical analysis confirmed phenylpropyl cinnamate as the predominant constituent in both sweetgum oil samples. This finding aligns with previous studies reporting cinnamate derivatives as the major bioactive components of *Liquidambar orientalis* extracts [27–29]. The pronounced activity observed at very low concentrations (0.001 µg/mL) is likely attributable to the high biological potency of cinnamate esters, which are known to

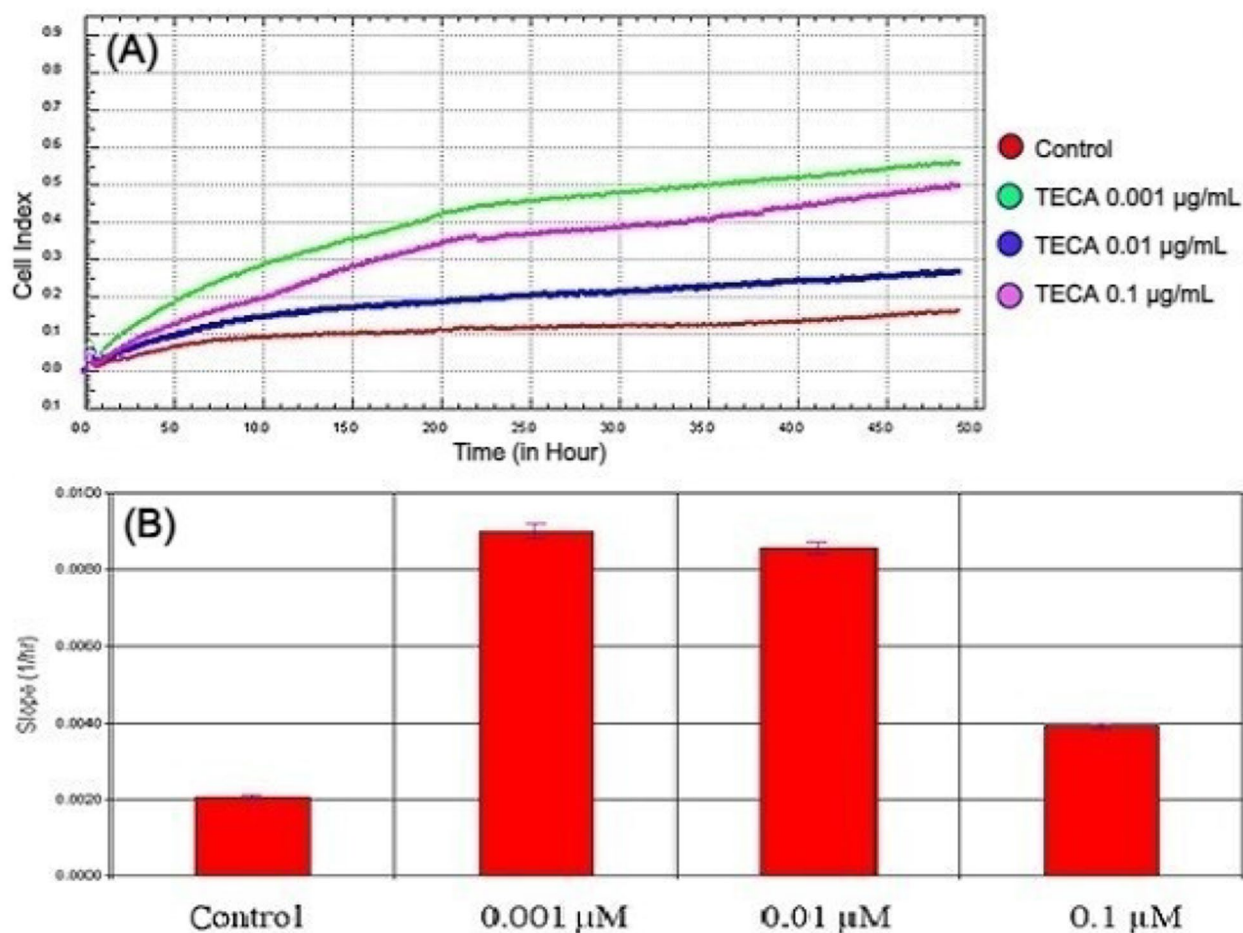


Fig. 7 **A** Graph of time-dependent migration effect of TECA concentrations on HaCaT keratinocyte cells in RTCA-DP ($n=6$); **B** Slope values of TECA concentrations in HaCaT keratinocyte cells in the RTCA-DP system after 72 h ($n=6$; mean $\pm$ standard deviation), $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. control. Data are mean $\pm$ SD ($n=6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. Control

modulate signalling pathways associated with cell proliferation and oxidative stress responses. Although direct causality cannot be established from the present in vitro data, the consistent predominance of phenylpropyl cinnamate across both samples, along with its known bioactivity, strongly suggests its major contribution to the observed effects. Nevertheless, other constituents—such as cinnamyl cinnamate, cinnamaldehyde, and benzyl alcohol—may exert complementary or synergistic effects, warranting further investigation.

The present study has certain limitations. First, only in vitro assays were conducted, and therefore the physiological relevance of the observed effects at very low concentrations remains to be confirmed in vivo. Second, the chemical composition of sweetgum oil can vary depending on geographic origin, harvest season, and extraction method, which may influence its biological activity. Third, although phenylpropyl cinnamate was identified as the dominant phytochemical, its specific contribution

to wound healing should be verified through studies using isolated compounds and mechanistic pathway analyses. Finally, no assessment was made regarding skin irritation or cytotoxicity at higher concentrations in advanced skin models, which is important for evaluating clinical safety.

Future studies should focus on in vivo wound-healing models to validate the efficacy and dose–response relationship of sweetgum oil. Mechanistic investigations are also needed to identify specific signaling pathways involved in keratinocyte proliferation and migration. In addition, pharmacological testing of phenylpropyl cinnamate alone, as well as in combination with other sweetgum constituents, would help clarify its precise role in mediating the observed effects. Comparative studies with established agents such as TECA could further define the relative therapeutic potential of sweetgum oil. Ultimately, clinical investigations will be essential to evaluate safety, tolerability, and efficacy in human subjects.

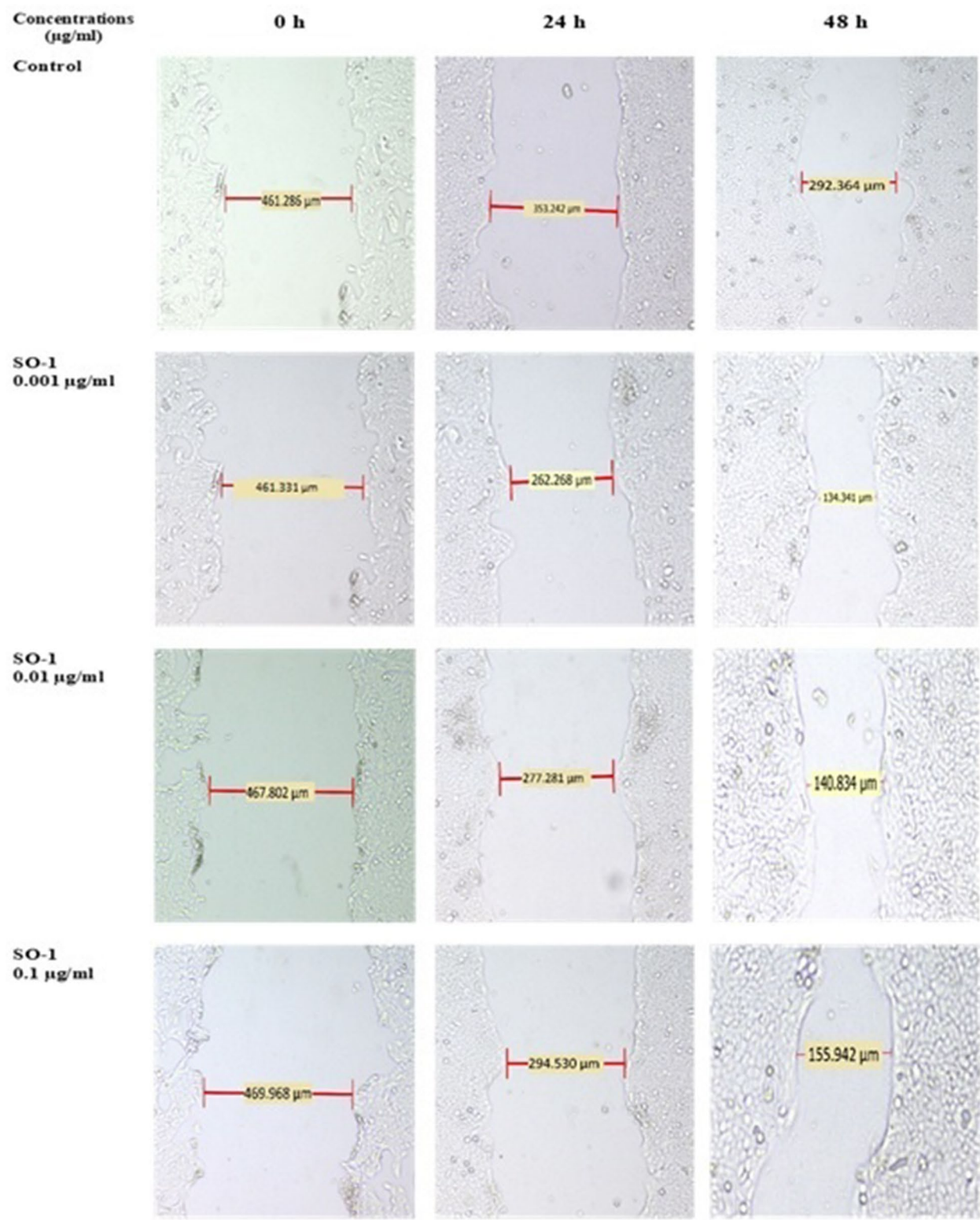


Fig. 8 Photomicrographs of the scratch assay 24 h and 48 h after the application of different concentrations of SO-1

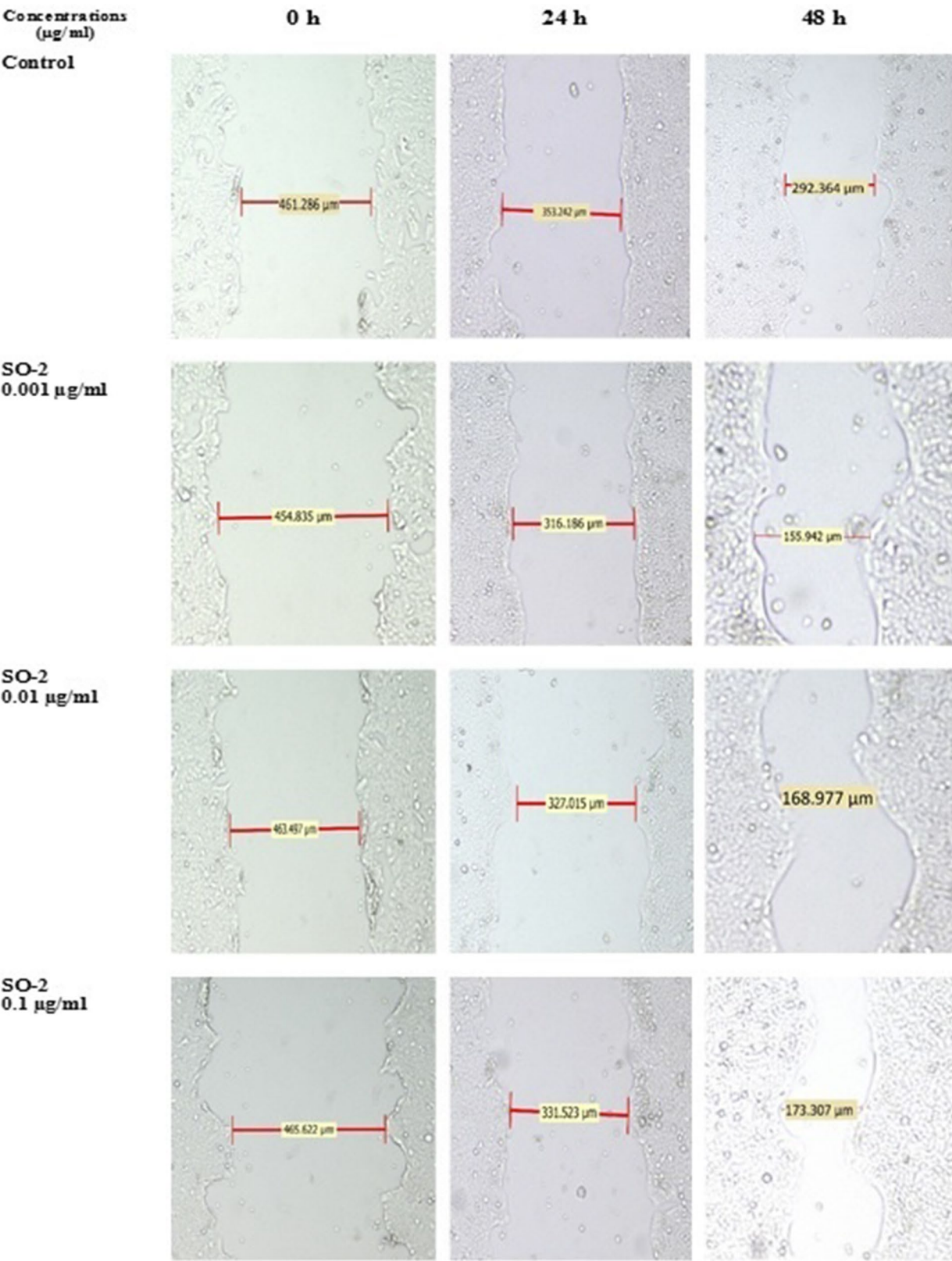


Fig. 9 Photomicrographs of scratch assay 24 h and 48 h after the application of different concentrations of SO-2

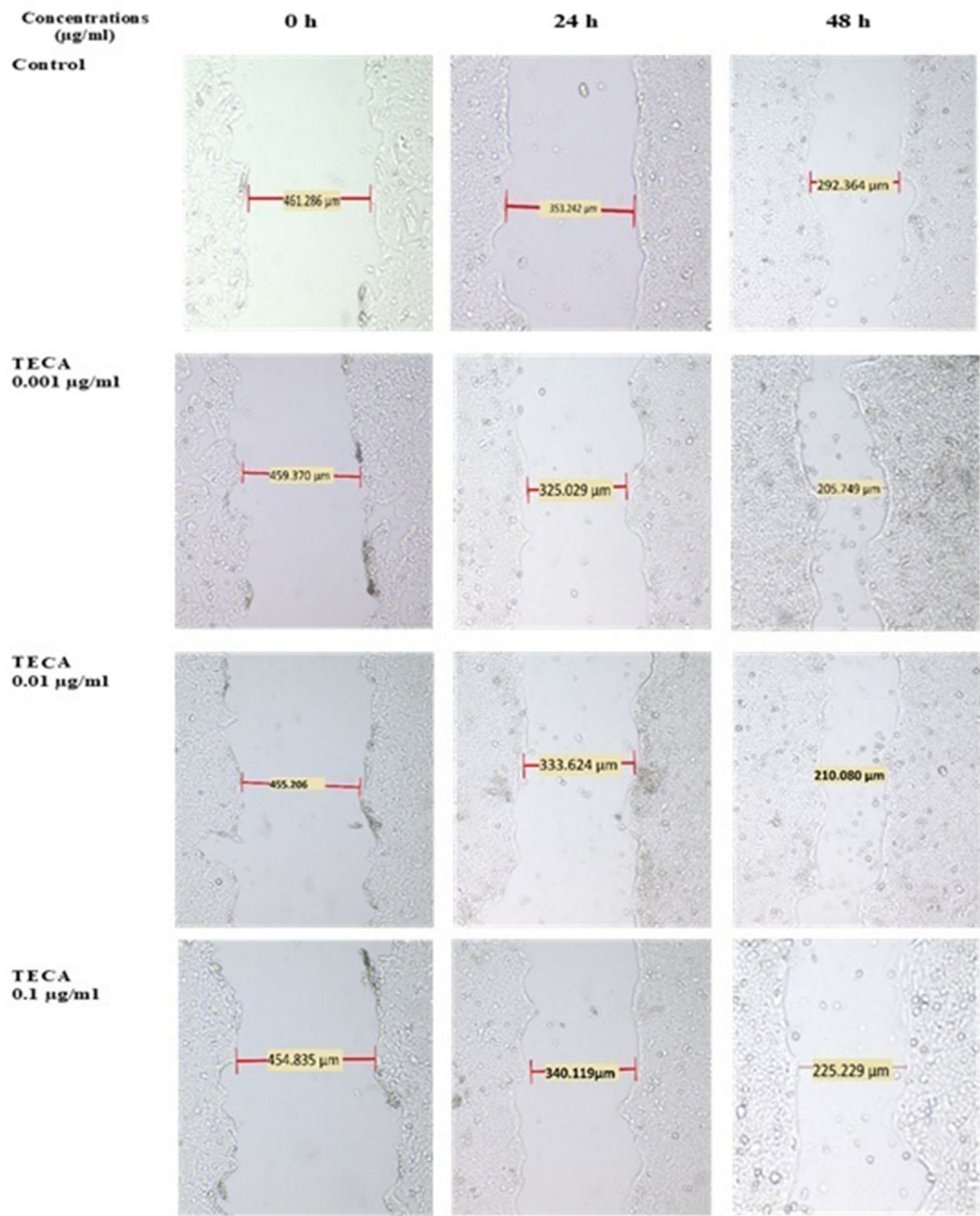


Fig. 10 Photomicrographs of scratch assay 24 h and 48 h after the application of different concentrations of TECA

Table 3 IT-TOF/MS high-resolution mass spectroscopy analyses of sweetgum samples, SO-1 and SO-2

Compound	Ionization Type	Adduct	Mass Calculated	Mass Found	Intensity for SO-1 (%)	Intensity for SO-2 (%)
Cinnamyl cinnamate	Positive	H	265.1223	265.1226	9.3	19.6
Phenylpropyl cinnamate	Positive	H	267.1380	267.1383	82.1	69.5
Benzyl alcohol	Positive	Na	131.0528	131.0499	2.1	3.1
Cinnamaldehyde	Positive	H	133.0648	133.0670	2.9	6.2
Cinnamic acid	Positive	H	149.0597	149.0584	0.2	0.9
<i>p</i> -hydroxy cinnamic acid	Negative	H	164.0401	163.0187	3.4	0.7

Conclusion

In conclusion, sweetgum oil promotes keratinocyte migration and proliferation at non-toxic concentrations, supporting its potential as a wound-healing agent. Phenylpropyl cinnamate appears to be an important contributor to this activity; however, its role remains speculative. These findings provide novel mechanistic insight and justify further in vivo validation.

Abbreviations

COL1A1	Collagen type I alpha 1
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum
HaCaT	Human keratinocyte cell line
IT-TOF/MS	Ion-trap time-of-flight mass spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
RTCA-DP	Real-time cell analysis dual plate system
RT-PCR	Real-time polymerase chain reaction
TECA	Titrated extract of <i>Centella asiatica</i>

Acknowledgements

The authors thank Anadolu University MERLAB facilities for technical support

Clinical trial number

Not applicable.

Authors' contributions

M.D. performed methodology, validation, formal analysis, and data curation. S.L. carried out software analysis and visualization. B.E. contributed to investigation and project administration. S.Ö. provided resources. M.Y. and Y.Ö. wrote the original draft and conducted review and editing. M.D. and Y.Ö. supervised the study. M.D. acquired funding.

Funding

This study was supported by Anadolu University Scientific Research Projects (Project No. 1705S306).

Data availability

All data generated or analysed during this study are included in this published article manuscript the last. Additional information can be provided by the corresponding author upon reasonable request.

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.

Competing interests

The authors declare no competing interests.

Received: 12 June 2025 / Accepted: 24 December 2025

Published online: 30 December 2025

References

- Law AL, et al. Comparison of Healthcare Costs Associated with patients receiving traditional negative pressure wound therapies in the post-acute setting. *Cureus*. 2020;12(11):e11790. <https://doi.org/10.7759/cureus.11790>.
- Vatankhah N, et al. Effect of systemic insulin treatment on diabetic wound healing. *Wound Repair Regen*. 2017;25:288–91. <https://doi.org/10.1111/wrr.12514>.
- Frasca D, Strbo N. Effects of obesity on infections with emphasis on skin infections and wound healing. *J Dermatol Skin Sci*. 2022;4(3):5–10. <https://doi.org/10.29245/2767-5092/2022/3.1157>.
- Maroz N, Simman R. Wound healing in patients with impaired kidney function. *J Am Coll Clin Wound Spec*. 2014;5:2–7. <https://doi.org/10.1016/j.jccw.2014.05.002>.
- Rawlings CR, et al. A rheumatology perspective on cutaneous vasculitis: assessment and investigation for the non-rheumatologist. *Int Wound J*. 2016;13:17–21. <https://doi.org/10.1111/iwj.12437>.
- Robson MC, et al. Randomized trial of topically applied repifermin (recombinant human keratinocyte growth factor-2) to accelerate wound healing in venous ulcers. *Wound Repair Regen*. 2001;9:347–52. <https://doi.org/10.1046/j.1524-475x.2001.00347.x>.
- Zarchi K. Wound healing and all-cause mortality in 958 wound patients treated in home care. *Wound Repair Regen*. 2015;23:753–8. <https://doi.org/10.1111/wrr.12335>.
- Hajhosseini B. Pressure injury. *Ann Surg*. 2020;271:671–9. <https://doi.org/10.1097/SLA.0000000000003567>.
- Franz, et al. Use of the wound healing trajectory as an outcome determinant for acute wound healing. *Wound Repair Regen*. 2000;8:511–6. <https://doi.org/10.1046/j.1524-475x.2000.00511.x>.
- Oryan A, et al. Burn wound healing: present concepts, treatment strategies and future directions. *J Wound Care*. 2017;26:5–19. <https://doi.org/10.12968/jowc.2017.26.1.5>.
- Vitale S, et al. Phytochemistry and biological activity of medicinal plants in wound healing: an overview of current research. *Molecules*. 2022;27(11):3566. <https://doi.org/10.3390/molecules27113566>.
- Baytop T. Therapy with Medicinal Plants in Turkey, in the Past and the Present [Turkish] 2nd ed, Nobel Tıp Basımevi, Istanbul, 1999.
- Budovsky A, et al. Effect of medicinal plants on wound healing. *Wound Repair Regen*. 2015;23:171–83. <https://doi.org/10.1111/wrr.12274>.
- Givol O, et al. A systematic review of *Calendula officinalis* extract for wound healing. *Wound Repair Regen*. 2019;27:548–61. <https://doi.org/10.1111/wrr.12737>.
- Korkmaz S, et al. Testing wound-healing activity in T15 fibroblast cultures: a morphometric analysis. *Altern Lab Anim*. 2000;28:41–51. <https://doi.org/10.1177/026119290002800107>.
- Öztürk N, et al. Wound-healing activity of St. John's wort (*Hypericum perforatum* L.) on chicken embryonic fibroblasts. *J Ethnopharmacol*. 2007;111:33–9. <https://doi.org/10.1016/j.jep.2006.10.029>.

17. Dikmen M. Evaluation of the wound healing potentials of two subspecies of *Hypericum perforatum* on cultured NIH3T3 fibroblasts. *Phytother Res.* 2011;25:208–14. <https://doi.org/10.1002/ptr.3243>.
18. Yusuf M, Aydin S. Relaxing actions of carvacrol on isolated rat duodenum smooth muscles: evidence for the role of potassium ion channels. *J Res Pharm.* 2024;28(5):1501–7. <https://doi.org/10.29228/jrp.828>.
19. Öztürk N, et al. Effects of gentiopicroside, sweroside and swertiamarine, seco-iridoids from gentian (*Gentiana lutea* ssp. *symphyandra*), on cultured chicken embryonic fibroblasts. *Planta Med.* 2006;72:289–94. <https://doi.org/10.1055/s-2005-916198>.
20. Hafizoğlu H. Analytical studies on the balsam *Liquidambar orientalis* Mill. by gas chromatography and mass spectrometry. *Holzforsch.* 1982;36:311–3.
21. Hansen MB, et al. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J Immunol Methods.* 1989;119:203–10. [https://doi.org/10.1016/0022-1759\(89\)90397-9](https://doi.org/10.1016/0022-1759(89)90397-9).
22. Van Meerloo J. Cell sensitivity assays: the MTT assay. *Methods Mol Biol.* 2011;731:237–45. <https://doi.org/10.1007/978-1-61779-080-5-20>.
23. Sağdıç O, et al. A study on inhibitory effects of sigla tree (*Liquidambar orientalis* Mill. var. *orientalis*) storax against several bacteria. *Phytother Res.* 2005;19:549–51. <https://doi.org/10.1002/ptr.1654>.
24. Özsel H, et al. Effects of oriental sweet gum storax on porcine wound healing. *J Invest Surg.* 2012;25:262–70. <https://doi.org/10.3109/08941939.2011.639847>.
25. Yanik ME. Comparison of topical treatment with silver sulfadiazine and sweet-gum oil (*Liquidambar orientalis*) on burn wound healing in an experimental rat model. *Anal Quant Cytol Histol.* 2016;38:168–74.
26. Deniz C. Comparison of sweetgum oil (*Liquidambar orientalis*), a plant resin, and ointments containing collagenase in wound healing [Turkish]. Kahramanmaraş Sütçü İmam University Faculty of Medicine, Department of General Surgery. Master thesis. Kahramanmaraş, 2010.
27. Lee YS, et al. Effect of plant essential oils and components from Oriental sweetgum (*Liquidambar orientalis*) on growth and morphogenesis of three phytopathogenic fungi. *Pestic Biochem Physiol.* 2009;93:138–43. <https://doi.org/10.1016/j.pestbp.2009.02.002>.
28. Park IK. Fumigant toxicity of Oriental sweetgum (*Liquidambar orientalis*) and valerian (*Valeriana wallichii*) essential oils and their components, including their acetylcholinesterase inhibitory activity, against Japanese termites (*Reticulitermes speratus*). *Molecules.* 2014;19:12547–58. <https://doi.org/10.3390/molecules190812547>.
29. Aşkun T. Anti-mycobacterial activity and chemical composition of essential oils and phenolic extracts of the balsam of *Liquidambar orientalis* Mill. (Altingiaceae). *Turk J Bot.* 2021;45:800–8. <https://doi.org/10.3906/bot-2109-37>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.