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Biological Activities and Gene Expression Effects of Blended Essential Oils in Promoting Dermal Wound Healing

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Abstract

Essential oils (EOs) are highly concentrated plant-derived compounds that have been used for centuries for their benefits in medicinal, food-processing, and commercial purposes - notably within the realm of cultural and luxury industries. However, only a single essential oil has been extensively studied for biological activities and wound healing. This study aimed to investigate the synergistic potential of blended essential oils (BEO) composed of tea tree essential oil (TTO), lavender essential oil (LEO), rosemary essential oil (REO), and turmeric essential oil (TEO) for its biological activities and wound healing effects on human dermal fibroblast (HDF) cells. BEO exhibited the strongest antioxidant activity against 2,2 diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals with IC_{50} values of 10.669 and 108.497 mg/mL, respectively. It exhibited the lowest cytotoxicity against Vero cells and also demonstrated promising anti-inflammatory and anti-collagenase activities. The *in vitro* scratch wound healing assay on human dermal fibroblast cells indicated that BEO significantly increased cell migration rate after 48 h of incubation. The gene expression study revealed that BEO promoted wound healing by upregulating the *COL1A1* gene, which is involved in type I collagen synthesis. This study is the first to confirm that the blended essential oil (BEO) has the significant potential for use as wound healing agent.

Keywords**:** *biological activities; blended essential oil; collagen gene expression; human dermal fibroblast; wound healing*

1. Introduction

A wound is generally defined as damage to the skin surface or other related tissues. There are two basic types of wounds, including reddish-raw open wounds and closed-dry wounds. Types of common open wounds are surgical incisions, abrasions, lacerations (blunt trauma wounds), puncture or penetrating wounds, burns-scalds, and ulcers. Surgical incisions, both incisional and excisional, involve cuts through the skin and soft tissues, post-procedure stitches may leave visible scars on the skin surface, especially if not properly cared for (Wachtel et al., 2022). Abrasions are superficial wounds that involve only the top layer of skin. They are often caused by friction or scraping and may be accompanied by minimal bleeding (Yen et al., 2024). Abrasions typically heal quickly and without complications. Lacerations are deeper wounds that involve the underlying tissue. They are often caused by sharp objects and may be accompanied by significant bleeding. Lacerations may require stitching or staples to close the wound and promote healing (Tsai et al., 2023). Puncture or penetrating wounds are caused by sharp objects that pierce the

skin. They are usually small and may not bleed much, but they can be deep and may damage underlying structures. Additionally, puncture wounds pose a risk of infection. (Nguyen et al., 2018). Burns and scalds are caused by heat, chemicals, or radiation. They can be classified as first-degree (superficial), second-degree (partial thickness), or third-degree (full thickness) depending on the depth of the injury. Burns can be very painful and can take a long time to heal. Ulcers are wounds that form on the skin or mucous membranes and are caused by a variety of factors, including poor circulation, diabetes, and pressure. They can be slow to heal and are at risk of infection (Wen et al., 2022).

Wound healing is a multifaceted and dynamic biological process involving coordinated interactions between tissues to restore skin integrity and homeostasis after an injury. This process plays a critical role in preventing infections and facilitating the repair of damaged tissues. Reactive oxygen species (ROS) serve a dual function in this cascade. At physiological levels, ROS act as essential signaling molecules, promoting key processes such as cell migration, cytokine activity, and angiogenesis through pathways like platelet-derived growth factor (PDGF) signaling. However, excessive ROS production results in oxidative stress, which causes damage to cellular components, including lipids, proteins, and nucleic acids. This oxidative damage disrupts cellular homeostasis, induces cytotoxicity, and significantly impairs the wound healing process (Liang et al., 2021; Sureda et al., 2023; Bahadur, & Fatima, 2024). To counteract oxidative stress and support the wound healing process, the topical application of compounds with free-radical-scavenging properties has proven effective. Among these, plant-derived bioactive compounds, long used in traditional medicine, have gained attention for their antioxidant and antibacterial properties. These phytochemicals promote tissue regeneration and protect against oxidative and microbial damage, making them valuable candidates for advanced wound care (Nascimento et al., 2022; Zhao et al., 2023).

Essential oils (EOs) are considered as an essential source of active agents. EOs are known for their natural wound healing properties, due to their antimicrobial, antioxidant, anti-inflammatory, and tissue regenerative properties (de Sousa et al., 2023; Bahadur, & Fatima, 2024). They have been reported as an effective role in the wound healing process (Nascimento et al., 2022). Several studies have investigated their biological activities related to the wound healing process. Tea tree essential oil (TTO), derived from the leaves of the tea tree *Melaleuca alternifolia*, is known as a promising agent for treating *Staphylococcus aureus*-infected wounds (Nguyen et al., 2023) which might be a result of terpenoids, the major constituents. TTO also accelerated the formation of granulation tissue and reduced inflammation (Zhao et al., 2023). Essential oil from *Lavandula angustifolia* (lavender), known as LEO, promotes collagen synthesis, fibroblast differentiation, and wound contraction due to its main constituents, including linalool, linalyl acetate, lavandulol, geraniol, bornyl acetate, borneol, terpineol, and eucalyptol (Liang et al., 2023; Todorova et al., 2023). In addition, rosemary essential oil (REO) harvested from the leaves of the rosemary plant *Rosmarinus officinalis* and predominated by 1,8-cineole can increase the skin flap survivability of rats (Li Pomi et al., 2023). Furthermore, turmeric essential oil (TEO) comes from the rhizomes of the turmeric plant *Curcuma longa* and is known for its highly-potent medicinal properties. The major constituents of TEO are curcuminoids which are mainly responsible for its anti-inflammatory, antioxidant, and anticancer properties (Aminnezhad et al., 2023). Curcuminoids are effective across multiple stages of wound healing, including reducing inflammation, promoting tissue formation, and supporting collagen remodeling (Kumari, & Nanda, 2023). Although there are some reports on biological activities and wound healing potential of these EOs, there is no efficacy studies for their synergistic effects in wound healing.

2. Objective

The main objective of this study was to evaluate the biological activities of individual essential oils (EOs), including TTO, LEO, REO, and TEO, as well as the blended essential oil (BEO). The most promising EO was then selected for further investigation of its *in vitro* wound-healing and gene expression efficacy compared to BEO.

3. Materials and Methods

3.1 Preparation of EOs

EOs of *Melaleuca alternifolia*, *Rosmarinus officinalis*, *Lavandula angustifolia* were provided by Chommpinn (Bangkok, Thailand). *Curcuma longa* L. was prepared from fresh plant materials. The fresh sample was then cut, mashed, and distillated using a steam distillation apparatus. Meanwhile, BEO was prepared by mixing these four EOs at the ratio of 1:1:1:1. The EO samples were stored in a dark glass

container at 4°C before analysis. Yields of 2.0, 0.8, 2.0, and 4.5% were for *Melaleuca alternifolia*, *Rosmarinus officinalis*, *Lavandula angustifolia*, and *Curcuma longa* L., respectively.

3.2 Antioxidant Activities by DPPH and ABTS Radical Scavenging Capacity Assays

The 2,2 diphemyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay was measured as described by Maduwanthi, & Marapana (2021) with some modifications. Briefly, 159 µL of EO samples were mixed well with 2,850 µL of DPPH radical solution (0.2 mg in ethanol). After incubating at room temperature in the dark for 30 minutes, the absorbance of the mixture was recorded at 517 nm using UV-Vis spectrophotometer. The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity assay was performed as reported by Re et al., (1999) with little alteration. Briefly, an ABTS solution was added to potassium persulfate solution, shaken intensively, and placed in a dark place at room temperature for 12-16 h. Then, 20 µL of EO samples were well mixed with 2,000 µL of ABTS solution and placed for approximately 6 min. The observed absorbance at 734 nm was recorded. Trolox solution was used to prepare a standard curve. DPPH and ABTS values of the samples were expressed in term of a milligram (mg) of Trolox equivalent antioxidant capacity (TEAC) per gram (g) of sample. Concentrations of samples required for inhibition of 50% of DPPH and ABTS radicals (IC_{50} values for DPPH and ABTS radicals) were obtained through extrapolation from regression analysis. The percentage of radical scavenging was calculated as follows:
Radical Scavenging (%) =

$$[(A_{\text{control group}} - A_{\text{test group}}) / A_{\text{control group}}] \times 100$$

3.3 Cytotoxicity Test by 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl Tetrazolium Bromide (MTT) Assay

Cytotoxicity percentage of EOs on Vero cell was determined using MTT assay as described by Hosseinzadeh et al., (2020) with some modifications. Briefly, Vero cells were seeded at a density of $1x10^4$ cells/well in 96-well microtiter tissue culture plates initially for 24 h at 37°C under 5% CO_2 and subsequently after the addition of EOs (62.5, 125, 250, 500, and 1,000 µg/mL), for another 48 h. After incubation, the medium was removed and 5 mg/mL of MTT was added. After incubation for 4 h, MTT solution was removed and 100 µg/well of solution (100% DMSO: 10% SDS at a ratio of 9 : 1) for dissolving formazan crystals was added. Cells in medium containing vehicles (1% DMSO) served as the control. The absorbance of sample giving dark purple color was measured at 570 nm using a microplate reader. Results were interpreted as percentage of cytotoxicity obtained by calculating the absorbance as follows:
Cytotoxicity (%) =

$$[(A_{\text{control group}} - A_{\text{test group}}) / A_{\text{control group}}] \times 100$$

3.4 *In vitro* Anti-inflammatory Activity

In vitro anti-inflammatory activity was assessed using the method described by Chandra et al., (2012) with some modifications. Briefly, reaction mixture (5 mL), consisted of 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS, pH 6.4), and 2 mL of different concentrations of EOs (0.625, 1.25, 2.5, 5, and 10 mg/mL). Then, the mixtures were incubated at 37°C ± 2 for 15 min. and heated at 70°C for 5 min. After cooling, the absorbance of the sample was measured at 660 nm. Diclofenac diethylammonium was used as a reference. The inhibition percentage of protein denaturation was calculated using the following formula:
Inhibition of protein denaturation (%) =

$$[(A_{\text{test group}} / A_{\text{control group}}) - 1] \times 100$$

3.5 Collagenase Inhibitory Assay

The collagenase inhibitory assay was measured as described by Thring et al., (2011) with slight modifications. Briefly, a mixture tube (1.7 mL) contained 5 µg of collagenase and 0.5 mg of PZ-peptide in 0.1 M tris buffer (pH 7.1) containing 20 mM CaCl2 in the presence of test EOs (0.001, 0.01, 0.1, and 1 mg/mL). Then, the tube was incubated at 37°C for 30 min., and 25 mM citric acid solution (1 mL) was added to terminate the reaction. After mixing with ethyl acetate (5 mL), the absorbance was measured at 320 nm. The inhibition percentage was calculated according to the following formula:
Collagenase inhibition (%) =

$$[(A_{\text{control group}} - A_{\text{test group}}) \times 100] / A_{\text{control group}}$$

3.6 *In vitro* Wound Healing Promoting Activity

Cell viability of Human dermal fibroblast (HDF) cells treated with TEO and BEO was investigated to find the suitable concentration of the EOs for testing wound healing. *In vitro* wound healing-promoting activity was performed as described by Muhammad et

al., (2013). HDF cells were seeded in 96-well plates at a concentration of $3x10^5$ cells/mL cultured in a fibroblast media containing 5% FBS and grown to confluent cell monolayer. The media were pipetted out and discarded. A small area was scratched using a 200 µl pipette tip and the cells were then rinsed with PBS to remove any loosened debris. Fibroblast media with 1 mg/mL EOs were replaced and the plates were incubated at 37°C and 5% CO_2. The distance between two layers of cells which was scratched by pipette tip was then inspected microscopically at 0, 24, 30, and 48 h, respectively. As the HDF cells migrate to fill the scratched area, images were captured with digital camera attached to the microscope and connected to a computer system. Quantitative evaluation of wound closure (%) was expressed as the percentage of the wound area covered by the cells.

3.7 Analysis of Gene Expression of Collagen Synthesis

The human dermal fibroblast (HDF) cells were seeded in each 6-well plate at $2x10^5$ cells/well and incubated in an incubator containing 5% CO_2 at 37°C. After overnight incubation, the cells were treated with 1 mg/mL of EOs and incubated for 24 h. Cells were digested with trypsin and centrifuged at 1,500 rpm for 3 min. DNA was extracted from cell pellets using NucleoSpin® RNA Plus kit and cDNA was synthesized from RNA template using ReverTra Ace™ qPCR RT Master Mix with gDNA Remover. Gene expression of the collagen type 1 chain gene (*COL1A1*) was analyzed using polymerase chain reaction (PCR) with specific primers. PCR products were loaded in 1.5% agarose gel. The gel was run at 100 volts for 30 min. and stained with 0.5 ug/mL of ethidium bromide for 20 min. Then the DNA fragment was visualized under ultraviolet light using GelDoc2000 (Bio-Rad, USA).

3.8 Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in SPSS version 17.0 (IBM). Differences in means between the designated evaluations were considered significant at $p < 0.01$ and $p < 0.05$.

4. Results and Discussion

4.1 Antioxidant Activities by DPPH and ABTS Assays

Antioxidant activities of single EO (TTO, LEO, REO, and TEO) and BEO were investigated by DPPH and ABTS assays as shown in Table 1. In this regard, DPPH has been widely used to assess the antioxidant capacity, which would indicate the scavenging activity of active constituents (Chen et al., 2023). ABTS assay is also used to test the antioxidant ability of compounds by reacting ABTS with compounds into colorless ABTS, which can be measured by utilizing spectrophotometry method (Elbouzidi et al., 2024). The study showed that all essential oil samples (TTO, LEO, REO, TEO, and BEO) could scavenge DPPH and ABTS free radicals. DPPH assay results revealed that BEO showed the highest degree of electron-donating capacity by 0.237± 0.001 mgTREA/g of sample with the lowest IC_{50} value at 10.669 ± 0.412 mg/mL when compared to other EOs. BEO also exhibited higher antioxidant activities than other single EOs against ABTS at 1.827 ± 0.004 mgTREA/g and the lower IC_{50} value at 108.497 ± 3.995 mg/mL. The lower IC_{50} values correspond to higher antioxidant activity of EOs (Itam et al., 2021). The antioxidant effect of BEO is attributed to the presence of active constituents in each EO, including TTO, LEO, REO, and TEO, which exhibit synergistic activities. The result also showed that TEO had higher antioxidant activity when compared to all single EOs. It could be assumed that TEO can play a major role on the synergistic activities in the blended essential oil. Curcumin, the most important and well-known compound in TEO, has been found to have the ability to scavenge free radicals and also inhibit the lipid peroxidation process (Sureda et al., 2023). Curcumin is mainly found in the root of the turmeric plant and is responsible for the yellow color of TEO. The percentage of curcumin in TEO can vary, but it is usually around 3-5%. TEO does contain a wider variety of volatile EOs that include sesquiterpenes, monoterpenes, and sesquiterpenoids. These oils are responsible for the aroma of TEO. The composition of the essential oils in TEO vary depending on the geographic location, soil quality, and method of extraction. Nevertheless, it is usually composed of sesquiterpenes such as beta-caryophyllene and germacrene D, and monoterpenes such as terpinene-4-ol and ar-curcumene (Zhang et al., 2023). Previous studies reported that TEO could serve as a hydrogen donor antioxidant and applied as a part of daily supplements to prevent oxidative stress that causes many degenerative diseases (Qiang et al., 2021). LEO contains polyphenols such as flavonoids, which have radical scavenging properties (Slighoua et al., 2022). Another study reported that TTO and REO revealed richness in oxygenated monoterpenes, representing 51.06% and 69.61% of the total oil composition, respectively, which possess strong antioxidant properties (Labib et al., 2019).

Table 1 Antioxidant activities of essential oils by DPPH and ABTS assays

Sample	DPPH		ABTS	
	mg TEAC/g of Sample	IC_{50} (mg/mL)	mg TEAC/g of Sample	IC_{50} (mg/mL)
TTO	0.068 ± 0.001^{d}	77.090 ± 0.569^{d}	0.277 ± 0.004^{d}	1,319.490 ± 16.238^{e}
LEO	0.073 ± 0.001^{d}	74.548 ± 0.099^{c}	0.089 ± 0.000^{d}	1,435.625 ± 8.993^{f}
REO	0.029 ± 0.002^{d}	167.780 ± 1.858^{f}	0.286 ± 0.002^{d}	1,181.259 ± 13.736^{d}
TEO	0.225 ± 0.002^{d}	11.058 ± 0.301^{b}	1.569 ± 0.008^{d}	178.045 ± 0.484^{b}
BEO	0.237 ± 0.001^{d}	10.669 ± 0.412^{b}	1.827 ± 0.004^{d}	108.497 ± 3.995^{b}
Ascorbic acid	1,307.478 ± 7.956^{a}	0.004 ± 0.000^{a}	1,442.060 ± 13.844^{a}	0.231 ± 0.002^{a}
Alpha-tocopherol	356.582 ± 1.247^{b}	0.012 ± 0.000^{a}	412.719 ± 4.169^{c}	0.634 ± 0.003^{a}
BHT	40.335 ± 1.069^{c}	0.095 ± 0.001^{a}	587.638 ± 8.275^{b}	0.575 ± 0.004^{a}

Each value represents the mean ± standard deviation of triplicates followed by different letters are significant different ($p < 0.05$), TTO = tea tree essential oil, LEO = lavender essential oil, REO = rosemary essential oil, TEO = turmeric essential oil, BEO = blended essential oils, BHT = butylated hydroxytoluene.

Table 2 Cytotoxic activity of essential oils against Vero cells

Sample	IC_{50} (µg/mL)
TTO	938.600 ± 0.200^{c}
LEO	898.183 ± 0.071^{b}
REO	915.700 ± 0.100^{b}
TEO	954.483 ± 0.126^{c}
BEO	888.763 ± 0.025^{b}
1% DMSO	2.580 ± 0.010^{a}

Each value represents the mean ± standard deviation of triplicates followed by different letters are significant different ($p < 0.05$), TTO = tea tree essential oil, LEO = lavender essential oil, REO = rosemary essential oil, TEO = turmeric essential oil, BEO = blended essential oils, BHT = butylated hydroxytoluene, DMSO = dimethyl sulfoxide.

4.2 Cytotoxicity Activities by MTT Assay

MTT assay was used to examine the cytotoxic effects of TTO, LEO, REO, TEO, and BEO against Vero cell. This method indirectly determines cell viability through the metabolic reaction between MTT and mitochondrial reductase in viable cell (Mićović et al., 2021). The results revealed that BEO exhibited lower cytotoxicity level at 888.763 ± 0.025 µg/mL than those of single EOs (Table 2). TEO demonstrated a negligible toxicity level at 954.483 ± 0.126 µg/mL just slightly above that of TTO at 938.600 ± 0.200 µg/mL. This means that while there is no significant difference between TEO and TTO in terms of cytotoxicity level, TEO comes with more natural benefits when scientifically compared to TTO. Therefore, TEO was selected to investigate the anti-inflammatory, anti-collagenase, wound healing efficacy compared to BEO. On the other hand, LEO exhibited cytotoxic effects against Vero cell. This is due to linalyl acetate which is responsible for higher cytotoxicity (Hareng et al., 2024).

4.3 Anti-inflammatory Efficacy

Anti-inflammatory efficacy of TEO and BEO was investigated by calculating the inhibition percentage of protein denaturation. The results showed that TEO and BEO both have anti-inflammatory effects *in vitro* (Table 3). These essential oils inhibited albumin degradation by 50% (IC_{50}) at concentrations of 9.40 ± 0.59 and 7.81 ± 0.49 mg/mL representing 0.09 and 0.10 times that of diclofenac diethylammonium which is an anti-inflammatory drug, with IC_{50} of 0.80 ± 0.01 mg/mL respectively. Interestingly, BEO, the combination of EOs (TTO, LEO, REO, TEO), exhibited the synergistic anti-inflammatory effect by each single EO. Therefore, the synergistic interaction of their active compounds may enhance the efficacy of anti-inflammatory. It is confirmed that the blended essential oil has a potent anti-inflammatory effect when compared with single essential oil. This is the first report of anti-inflammatory activity by blended essential oils. TEO may play the main role on anti-

inflammatory effect in BEO. Several reports revealed that *in vivo* and *in vitro* studies of TEO can inhibit the production of pro-inflammatory cytokines such as TNF-α and IL-6, and also reduce paw edema in a carrageenan-induced inflammation model. The anti-inflammatory effect of TEO is thought to be due to the presence of curcumin, which has been found to inhibit the activity of enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) involved in inflammation (Dehzad et al., 2023). Furthermore, curcumin regulates inflammatory signaling pathways and inhibit the production of inflammatory mediators (Peng et al., 2021).

4.4 Anti-collagenase Property

Anti-collagenase property was performed using spectrophotometric method. Collagenase is responsible for breaking down the skin structure leading to skin ageing (Chaudhary et al., 2019). BEO inhibited collagenase with IC_{50} of 0.11 ± 0.01 mg/mL, which is 0.91 times higher than that of vitamin C, which demonstrates IC_{50} of 0.10 ± 0.01 mg/mL (Table 3). The anti-collagenase property of BEO might be due to the synergistic effect of active constituents of each EO. It could be assumed that BEO could enhance collagen synthesis due to the synergistic action of all constituents in the combined oils. However, the activity of TEO slightly inhibited collagenase. Previous findings reported that curcumin in TEO has been found to inhibit matrix metalloproteinases (MMPs) including collagenase which are responsible for collagen breakdown resulting in skin ageing. Curcumin also increased cellular proliferation and collagen synthesis at the wound skin site (Kumari et al., 2022). This confirms that BEO does not suppress collagen as one vital constituent to promote skin healing and remodeling post wound trauma (Wu et al., 2023).

4.5 *In vitro* Wound Healing

Cell viability of human dermal fibroblast (HDF) cells treated with TEO and BEO was investigated to find the non-cytotoxic concentration for testing wound healing. As shown in Table 4, TEO and BEO were non-toxic to dermal fibroblast cells at concentrations of 0.0001 - 1 mg/mL with having cell survival percentages between 95.10% - 103.39% and 94.26% - 103.88%, compared to those of vitamin C at 81.26% - 102.48%, respectively. The result revealed that the percentage of cell viability of TEO and BEO were significantly higher than those of vitamin C. *In vitro* wound healing efficacy of TEO and BEO was investigated using wound scratch test assay. TEO and BEO at a concentration of 1 mg/mL exhibited astringent effect. Dermal fibroblast cells exposed to BEO reached 50% wound closure at approximately 24 h after scratching. At 48 h after wound creation, wounds had reached 80% confluence (Figure 1). It was capable to stimulating dermal fibroblast cells to migrate towards each other faster than the control group (10% DMSO and DMEM) but slower than vitamin C at a concentration of 1 mg/mL throughout the 48-hour test period (Figure 2). It is assumed that the active compounds found in BEO play an important role in wound healing process. One of the main active constituents in the combined oil is curcuminoids of TEO. The previous report revealed both incisional and excisional open-types of wounds can be treated and intervened by solely utilizing curcuminoids (Yen et al., 2018). Therefore, the result suggests that this is the first report on wound healing of blended essential oil combined with tea tree essential oil, lavender essential oil, rosemary essential oil, and turmeric essential oil.

4.6 Gene Expression of Collagen Synthesis

The expression of *COL1A1*, the collagen type I alpha 1 chain gene, was determined by treating HDF cells in the presence of 0.1 mg/mL of TEO and BEO for 24 h. The results showed that TEO and BEO at the concentration of 1 mg/mL increased *COL1A1* expression by 1.27-fold and 1.51-fold, respectively. Additionally, the difference between the TEO and BEO induced gene expression was statistically significant when compared with untreated control ($p > 0.01$) (Figure 3). In our study, we first examined the effect of blended essential oils containing four essential oils including tea tree essential oil, lavender essential oil, rosemary essential oil, and turmeric essential oil on collagen synthesis. The BEO confirmed that collagen synthesis in dermal fibroblasts increased when compared with the control. TEO also showed the comparable effect on gene expression of type 1 collagen synthesis. It is known that type I collagen is the most common type of collagen in the human body, consisting of a type I collagen α 1 chain encoded by the *COL1A1* gene (Henriksen, & Karsdal, 2024). Our finding is consistent with previous study that curcumin in TEO promotes gene expression of collagen type I in the *in vitro* wound healing model (Rujirachotiwat, & Suttamanatwong, 2021).

Table 3 Comparative anti-inflammatory and anti-collagenase effects of TEO and BEO

Sample	Anti-Inflammatory, IC_{50} (mg/mL)	Anti-Collagenase, IC_{50} (mg/mL)
TEO	9.40 ± 0.59^{b}	$>1{,}000^{b}$
BEO	7.81 ± 0.49^{b}	0.11 ± 0.01^{a}
Diclofenac diethylammonium	0.80 ± 0.01^{a}	-
Vitamin C	-	0.10 ± 0.01^{a}

Each value represents the mean ± standard deviation of triplicates followed by different letters are significant different ($p < 0.05$), TEO = turmeric essential oil, BEO = blended essential oils.

Table 4 Cell viability of dermal fibroblast cells treated with TEO and BEO

Sample	Cell Viability (%)				
	0.0001 mg/mL	0.001 mg/mL	0.01 mg/mL	0.1 mg/mL	1 mg/mL
TEO	103.39 ± 3.83^{a}	101.42 ± 4.08^{b}	99.67 ± 2.11^{b}	98.12 ± 6.23^{b}	95.10 ± 3.85^{a}
BEO	103.88 ± 2.04^{a}	102.19 ± 2.72^{a}	101.63 ± 5.78^{a}	98.61 ± 3.29^{b}	94.26 ± 4.09^{b}
Vitamin C	102.48 ± 3.05^{b}	100.55 ± 2.73^{c}	99.59 ± 6.09^{b}	99.20 ± 1.74^{a}	81.26 ± 5.78^{c}

Note: Each value represents the mean ± standard deviation of triplicates followed by different letters are significant different ($p < 0.05$), TEO = turmeric essential oil, BEO = blended essential oils.

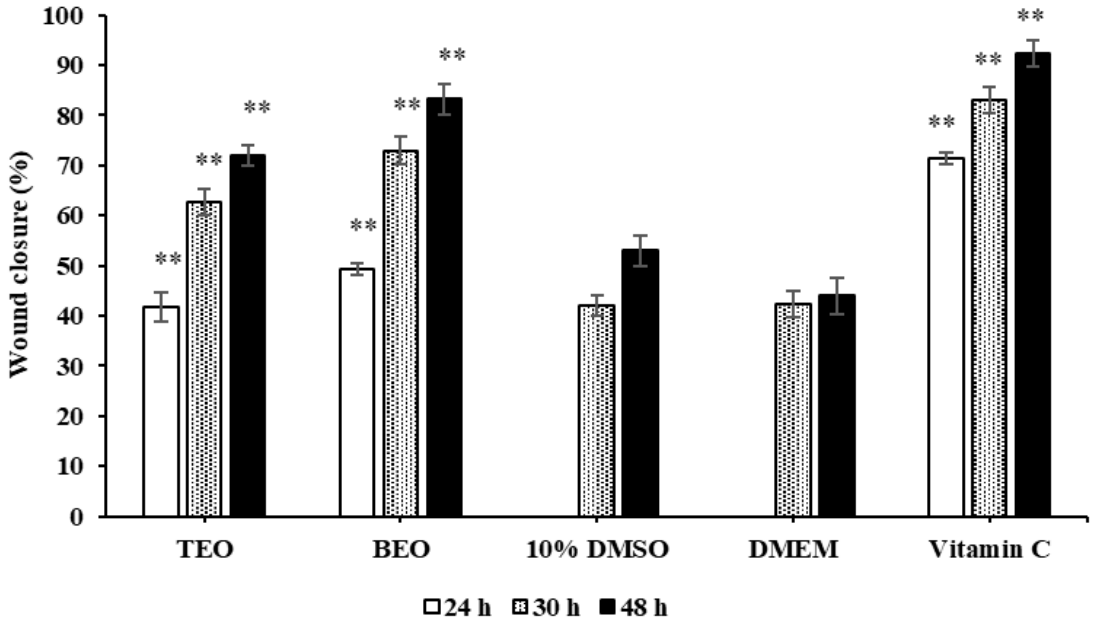

Figure 1 Wound closer of dermal fibroblast cells after the scratching. Results are presented as mean values ± SD of three independent experiments. (**P < 0.01). Note: TEO = turmeric essential oil, BEO = blended essential oils, DMSO = dimethyl sulfoxide, DMEM = dulbecco's modified eagle medium

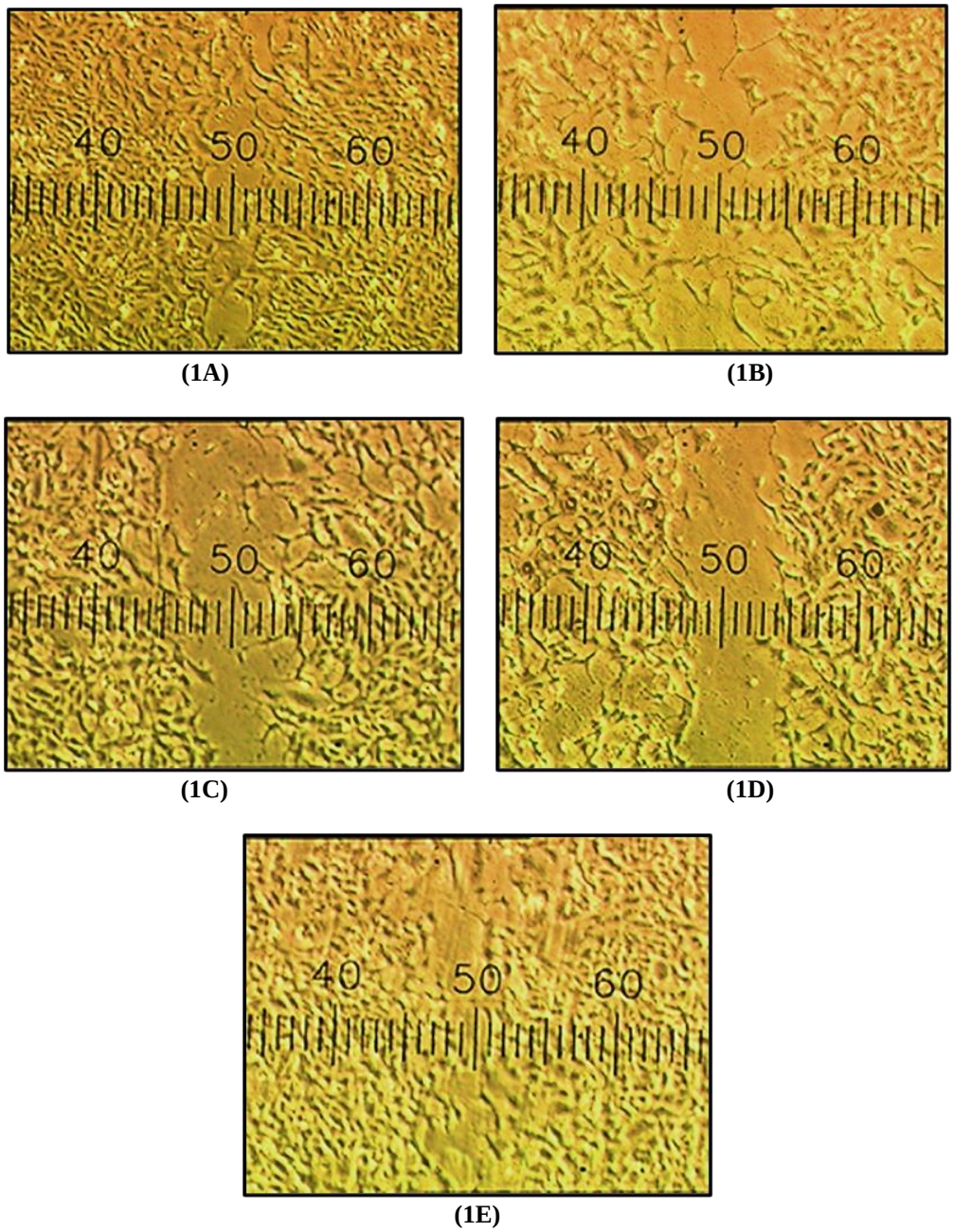

Figure 2 Migration of dermal fibroblast cells at the scratch area after 48 h treatment. 1A-1E represent BEO, TEO, Control (10% DMSO), Control (DMEM), and Vitamin C treatments, respectively

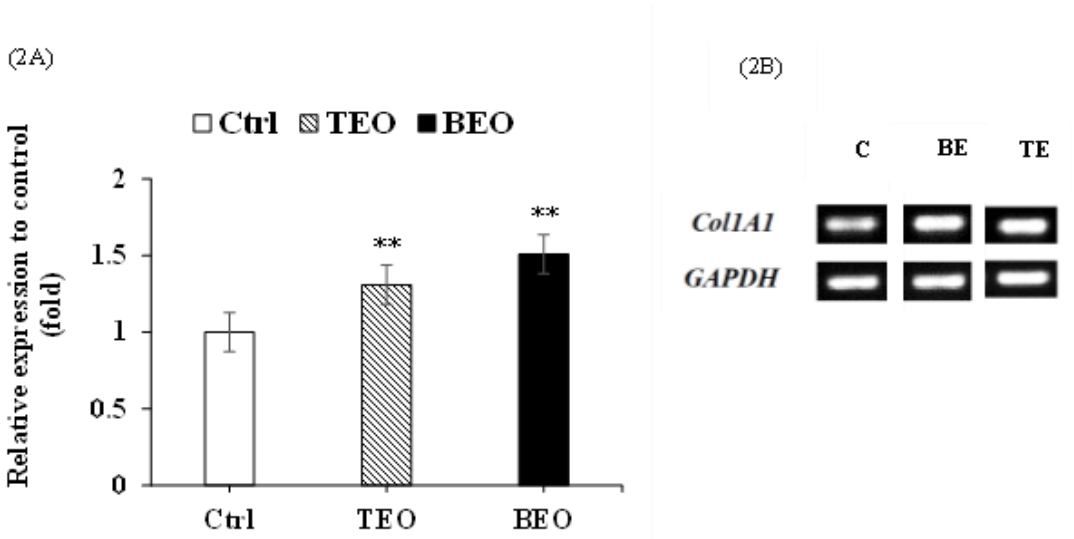

Figure 3 The expression of *COL1A1* in human dermal fibroblast cells in response to TEO and BEO examined by RT-PCR (A) and gel electrophoresis (B). Results are presented as mean values ± SD of three independent experiments. (**P < 0.01).
Note: Ctrl = control, TEO = turmeric essential oil, BEO = blended essential oils

5. Conclusion

BEO exhibited the highest antioxidant activity by scavenging DPPH and ABTS free radicals and demonstrated lower toxicity to Vero cells. The *in vitro* anti-inflammatory effect of BEO was greater than that of diclofenac diethylammonium, an anti-inflammatory drug. BEO could inhibit collagenase. Wound healing efficacy of BEO consisting of tea tree essential oil, lavender essential oil, rosemary essential oil, and turmeric essential oil was investigated for the first time. BEO increased gene expression of *COL1A1* which is responsible to enhancing collagen synthesis due to the synergistic action of all constituents in the combined oils. Therefore, BEO could be a potential agent for wound healing. Further *in vivo* study is needed to confirm the wound healing of the combined oil.

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