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In vitro Wound Healing Potential of Petroleum Ether Extracts from *Litsea garciae* Fruit Pulp and Seeds

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ABSTRACT

This study explores *Litsea garciae*'s (*L. garciae*) untapped wound healing potential, despite its traditional medicinal use. The current study aimed to elucidate the essential role of lipids in the wound healing process by evaluating the effects of *L. garciae* petroleum ether (PE) lipid extracts on human dermal fibroblast (HDF) cell line. The cytotoxicity of PE lipid extracts on the HDF was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. An *in vitro* study on the migration of HDF was performed using the wound scratch assay while the chemotactic motility of HDF was conducted by the transwell assay. The effects of *L. garciae* extracts on the expression levels of collagen type I and epidermal growth factor (EGF) were assessed using the enzyme-linked immunosorbent assays (ELISA). The MTT assay showed that PE lipid extracts (for pulp, 7.5 – 250 µg/mL and for seed, 7.5 to 500 µg/mL) were non-cytotoxic to HDF cells. Both extracts promoted cell migration, with 31.25 µg/mL pulp extract exhibited the highest wound closure rate (91.27 ± 8.35%) at 24 h, and 7.5 µg/mL seed extract significantly enhanced closure (90.62 ± 3.12%). The transwell assay indicated increased chemotactic motility when the HDF cells were treated with lipid extracts. Collagen type I synthesis improved in both pulp and seed lipid extracts. The pulp extracts enhanced EGF secretion at various concentrations, while the seed extracts showed no improvement. The study highlights *L. garciae* extracts' potential in wound healing by facilitating cell migration, collagen synthesis, and EGF secretion.

Keywords: Collagen type I, EGF, *Litsea garciae*, petroleum ether extract, wound healing

INTRODUCTION

A wound is formed when the skin layers are damaged thus disrupting the skin structure and protective barrier. Upon disruption of the skin layers' function (Lazarus *et al*., 1994), wound healing response with a cascade of events to restore damaged tissue to the normal anatomical function and structure. Wound repair is a highly regulated and intricate biological process to replace and reestablish impaired tissues. There are numerous treatments and medication options for wound healing that are available in the market, especially synthetic drugs such as antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs). However, long-term use of antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) is often associated with adverse effects such as gastrointestinal irritation, antibiotic resistance, delayed wound healing, and allergic reactions. Hence, there is still a necessity to research new effective medicine, particularly those derived from medicinal plants. The popularity of researching medicinal plants could be due to the perception that they cause minimal adverse side effects and are better tolerated by the body due to their natural bioactive compounds and antioxidant properties.

Litsea garciae (*L. garciae*) is known locally as engkala in Sarawak, Malaysia. *L. garciae* is rich in nutrients (fatty acids, phytochemicals, phenolic and flavonoid contents) and has many pharmacological properties (antioxidants, antimicrobial, antifungal, anticancer and anti-inflammatory) (Amit & Ling, 2021). The pulp and seed of *L. garciae* contain various minerals, including calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, vitamin C and zinc. Additionally, *L. garciae* contains various phytochemical components such as alkaloids, amides, butenolide, flavonoids, lignans, steroids and terpenes (Wulandari *et al.*, 2018). Besides, *L. garciae*

seed oil is particularly abundant with lauric acid and oleic acid, the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) respectively (Poli & Assim, 2019; Ling *et al.*, 2022). The petroleum ether lipid extracts of *L. garciae* contain a mixture of fatty acids (Ling *et al.*, 2022). The major component of saturated fatty acids (SFA) of *L. garciae* seed oil (w/w) are lauric acid (75.31%), and palmitic acid (7.39%). While the unsaturated fatty acids (UFA) are mainly oleic acid (7.73%) and linoleic acid (2.71%). On the other hand, the SFA of *L. garciae* lipid pulp extracts contain small amount of lauric acid (0.11%) but rich with palmitic acid (33.05%) and stearic acid (8.97%). The UFA of pulp extracts contain high amount of oleic acid (40.73%) and linoleic acid (7.21%).

Lipids play an important role in regulating complex wound healing process. Lipids aid in maintaining structural integrity of cell membrane during wound repair (de Albuquerque *et al.*, 2023). In addition, lipids supply energy for the proliferation and remodelling of wounded cells (Souto *et al.*, 2021). Moreover, lipids such as PUFAs act as precursors for the synthesis of eicosanoids, which mediates intercellular signalling (Pils *et al.*, 2021). Omega-6 fatty acids have been demonstrated to regulate proliferation, migration and inflammatory mediators' synthesis of cells. Linoleic acid aids in the modulation of proinflammatory mediators in the inflammatory phase (Silva *et al.*, 2018). Furthermore, according to Aoki *et al.* (2019), the derivatives of lipids improve inflammatory cells recruitment to the wound sites and promote angiogenesis. Besides, research by Ling *et al.* (2022) discovered that Gram-positive bacteria and Gram-negative bacteria are susceptible to *L. garciae* lipid pulp and seed extracts, where the extracts exhibit antimicrobial activities towards some bacteria. Such antimicrobial properties are advantageous for promoting wound healing.

Previous reports have highlighted the bark, leaf and fruit of *L. garciae* possessed properties of anti-inflammatory, antioxidant, anticancer, antifungal and antimicrobial (Johnny *et al.*, 2010; Kutoi *et al.*, 2012; Hassan *et al.*, 2013; Wulandari *et al.*, 2018; Raduan *et al.*, 2022). The indigenous people in Sarawak claimed that the leaves, shoots and barks of *L. garciae* had wound healing effects when these plant parts were used as wound healing dressings (Mirfat *et al.*, 2018). However, there was no data on the *L. garciae* fruit (pulp and seed) wound healing potential. Since lipids play crucial roles in wound healing and have some antimicrobial properties, this study is the first to scientifically validate the wound healing effects of lipid extracts from the pulp and seed of *L. garciae* using *in vitro* fibroblast assays. This study used petroleum ether for extraction since it is a non-polar solvent and thus able to extract the lipophilic lipid compounds (fatty acids, sterols, and other hydrocarbons). In this current research, the *L. garciae* lipid pulp and seed extracts potential wound healing effects were investigated through the *in vitro* studies on the migration and chemotactic motility of fibroblasts and their abilities in stimulating collagen type I and epidermal growth factor (EGF) secretion.

MATERIALS AND METHODS

Preparation of Petroleum Ether Lipid Extracts of *L. garciae*

Litsea garciae fruits were obtained from Sarawak state of Malaysia. The pulp and seed of *L. garciae* fruits were separated, lyophilised and ground into fine powder. The total lipid was extracted with a Soxhlet extractor by adding 300 mL of petroleum ether (PE) to 30 g of the *L. garciae* pulp and seed powder at 60 °C for 9 hours. The PE lipid extracts of *L. garciae* were obtained after the evaporation of PE by using a rotary evaporator (vacuum pressure at 332 hPa and heating bath temperature at 35 °C). The remaining PE in the lipid extracts was removed under a stream of nitrogen gas. The total lipids were then kept at -20 °C storage until further use.

Cell Culture

The human dermal fibroblast (HDF) cell line (cell name: OUMS-36T-4F; reference number: JCRB1006.4F) was acquired from the National Institutes of Biomedical Innovation, Health and Nutrition, Japan. The culture of HDF cells were performed in growth medium (Dulbecco's modified Eagle medium with 10% FBS and 1% penicillin-streptomycin mixed solution). The cells were incubated in a humidified incubator at 37 °C, 95% air and 5% CO_2. The assays were performed when the cells reached 80% confluency.

Cytotoxicity Assay

Cytotoxic effects for the PE lipid pulp and seed extracts of *L. garciae* were assessed by MTT assay (Bolla *et al.*, 2019; Li *et al.*, 2019). Seeding of HDF cells was conducted in a 96-well plate, at 2.2×10^4 cells/well in growth medium (200 µl). The cells were cultured for 24 h before adding different concentrations of PE lipid pulp and seed extracts (7.5, 15, 31.25, 62.5, 125, 250 and 500 µg/mL). After 24 h of incubation, the used growth medium was aspirated and washing of cells was performed using Dulbecco's phosphate buffered saline (DPBS). Next, addition of 20 µl 5 mg/mL MTT solution and fresh growth medium (180 µl) into the wells was conducted prior to the incubation for 3 h. The decantation of supernatant was performed before adding 100 µl of DMSO to solubilise the purple formazan crystals. Then, the plate was placed in an orbital shaker at 37 °C for 15 min. Using a microplate reader, measurement of absorbance at 570 nm was conducted. DMSO served as a blank while negative control was the untreated cells which was considered 100% viable. The experiment was repeated six times, and the presentation of data was as mean ± SD (n = 6). The calculation for cell viability percentage is shown in the equation below.

The calculation for cell viability percentage is, Eq. (1):

$$\% \text{ cell viability} = \frac{\text{Mean absorbance of test sample}}{\text{Mean absorbance of negative control}} \times 100\% \quad \text{Eq. (1)}$$

Wound Scratch Assay

The effect of PE lipid pulp and seed extracts on HDF cell migration was evaluated using scratch assay (Ado *et al.*, 2013; Buranasukhon *et al.*, 2017). The culture of HDF cells (1×10^5 cells/well) in a 12-well plate was performed with 1 mL/well of growth medium for 24 h. Next, to scratch the cell monolayer, a 200 µl sterile pipette tip was used. After scratching, used growth medium was aspirated. The washing of cells using DPBS was carried out to eliminate detached cells. Subsequently, 1 mL of serum-reduced medium (5% of serum) containing PE lipid pulp and seed extracts (7.5, 15 and 31.25 µg/mL) were used to treat the cells for 24 hours. The cells treated with 125 µg/mL allantoin served as a positive control while for the untreated cells, the cells were supplemented with serum-reduced medium as a negative control. The scratched area was monitored, and wound closure images were captured at 0, 6, 12 and 24 h intervals using a digital camera connected to an inverted microscope. The wound closure area was then measured using the digital camera's built-in software (Nikon NIS-Elements). The experiments were performed in triplicate and calculation for percentage of wound closure was presented by the equation stated below.

The calculation for percentage of wound closure is, Eq. (2):

$$\% \text{ Wound closure} = \frac{W_{oh} - W_{xh}}{W_{oh}} \times 100\% \quad \text{Eq. (2)}$$

W_{0h} = Wound at 0 hour
W_{xh} = Wound at 'x' hour; 'x' = 0, 6, 12, 24 and 48 hours

Transwell Assay

The chemotactic mobility of PE lipid pulp and seed extracts on HDF cells was assessed using the transwell assay (Li *et al.*, 2019; Mapoung *et al.*, 2021). Addition of 1 mL of serum-starved cell suspension (3×10^5 cells/well) to the upper chamber was conducted and 2 mL of 31.25 µg/mL of PE lipid pulp and seed extracts (diluted with growth medium) were added to the bottom chamber. Two negative controls were used: growth medium and serum-free medium (SFM). This approach was necessary as FBS can act as a chemoattractant that enhances cell migration (Zhang *et al.*, 2016). Therefore, the use of two negative controls could verify that the enhancement in the HDF cells' chemotactic motility was solely due to the PE lipid. After 24 h incubation, the removal of unmigrated cells on the upper membrane surface with a cotton swab was performed and the fixing of migrated cells on the lower surface of the membrane was done using 4% paraformaldehyde solution for 15 min at room temperature. Next, the washing of membrane using DPBS was conducted and the cells were stained with 0.2% crystal violet solution for 10 min. Next, staining solution was removed thoroughly with distilled water. The images of stained cells were captured under phase-contrast microscopy and the calculation for the number of migrated cells was done.

Collagen Type I ELISA

The effect of PE lipid pulp and seed extracts on the collagen type I secretion by HDF cells was

determined using ELISA. The culture of HDF cells was performed in a 12-well plate (1×10^5 cells/well) in serum-reduced medium (5% FBS) and incubated for 24 h before adding 1 mL of PE lipid pulp and seed extracts at 7.5, 15 and 31.25 µg/mL. The cells treated with 125 µg/mL of allantoin served as a positive control while incubation of untreated cells in serum-reduced medium served as a negative control. The collected cell supernatants were centrifuged at 1000 rpm, 4 °C for 5 min after incubation for 24 h. The supernatants were diluted with the sample diluent in a ratio of 1:200. The measurement of collagen type I secretion level in the supernatants was carried out using human Pro-Collagen I alpha 1 SimpleStep ELISA® Kit (ab210966) (Abcam, UK) following the manufacturer's instructions.

EGF ELISA

The expression of epidermal growth factor (EGF) by HDF cells treated with PE lipid pulp and seed extracts was detected using ELISA (Li *et al*., 2019). HDF cells were cultured at 1×10^5 cells/well in a 12-well plate. After 24 h of incubation, treatment of cells with 1 mL of PE lipid pulp and seed extracts at 7.5, 15 and 31.25 µg/mL was performed in serum-reduced medium (5% FBS). The cells treated with 125 µg/mL of allantoin served as the positive control. For the negative control, the untreated cells were incubated with serum-reduced medium. Cell supernatants were collected and centrifuged at 1000 rpm, 4 °C for 5 min after incubation for 24 h. The supernatants were then diluted with the sample diluent buffer at a ratio of 1:2. The measurement level of EGF expression in the cell supernatants was conducted using Human EGF ELISA Kit (RAB0149) (Sigma-Aldrich, USA) following the manufacturer's instructions.

Statistical Analysis

All assays were performed at least in triplicate and the data were presented as mean ± standard deviation of the mean (SD). Statistical analysis was carried out using the Statistical Package for the Social Science (SPSS). Comparisons among treatments were performed using a one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Different (HSD) test. Significance was set at $p<0.05$, where the p-value less than 0.05 indicated statistically significant.

RESULTS

Cytotoxicity of *L. garciae* Lipid Extracts

This experiment determined non-cytotoxic concentrations of the plant extracts for subsequent wound healing experiments. The concentration of extracts that was considered as toxic are those causing a 20 % reduction in cell viability compared to control. At 500 µg/mL, the PE lipid pulp extract exhibited mild cytotoxicity, reducing the cell viability to 80.15 ± 0.04% (Figure 1a). The PE lipid pulp extract with concentrations ranging from 7.5 to 250 µg/mL were non-cytotoxic with viabilities ranging from 85.48 to 101.84%. Similarly, PE lipid seed extracts at concentrations of 7.5 to 500 µg/mL showed no cytotoxicity and maintained cell viabilities between 85.65% and 106.10% (Figure 1b). Following the cytotoxicity assessment, concentrations of lipid extracts with higher cell viabilities (7.5, 15 and 31.25 µg/mL) were selected for further experiments. In addition, 0.05% DMSO used for dissolving the lipid and 125 µg/mL allantoin were non-toxic to the cells.

Effects of *L. garciae* Lipid Extracts on HDF Cell Migration

Cell migration is crucial for an effective healing of wound and evaluation of the effects of PE lipid pulp and seed extracts on cell migration was performed using a scratch assay. After 6 hours of incubation, all concentrations of PE lipid pulp extracts (Figure 2) showed enhanced cell migration rates. For instance, incubation with 31.25 µg/mL of PE lipid pulp extract resulted in a closure rate of 19.38% ± 4.73%, compared to 10.06% ± 4.15% for the negative control. Similar enhancements were observed at 12 hours and 24 hours of incubation. Similarly, PE lipid seed extracts (Figure 2) also improved cell migration rates at all tested concentrations. For instance, incubation with 7.5 µg/mL of PE lipid seed extract showed the highest wound closure rate (22.28% ± 1.24%) compared to the negative control (10.06% ± 4.15%) at 6 hours (Figure 3). This improvement was also observed at the incubation for 12 and 24 hours.

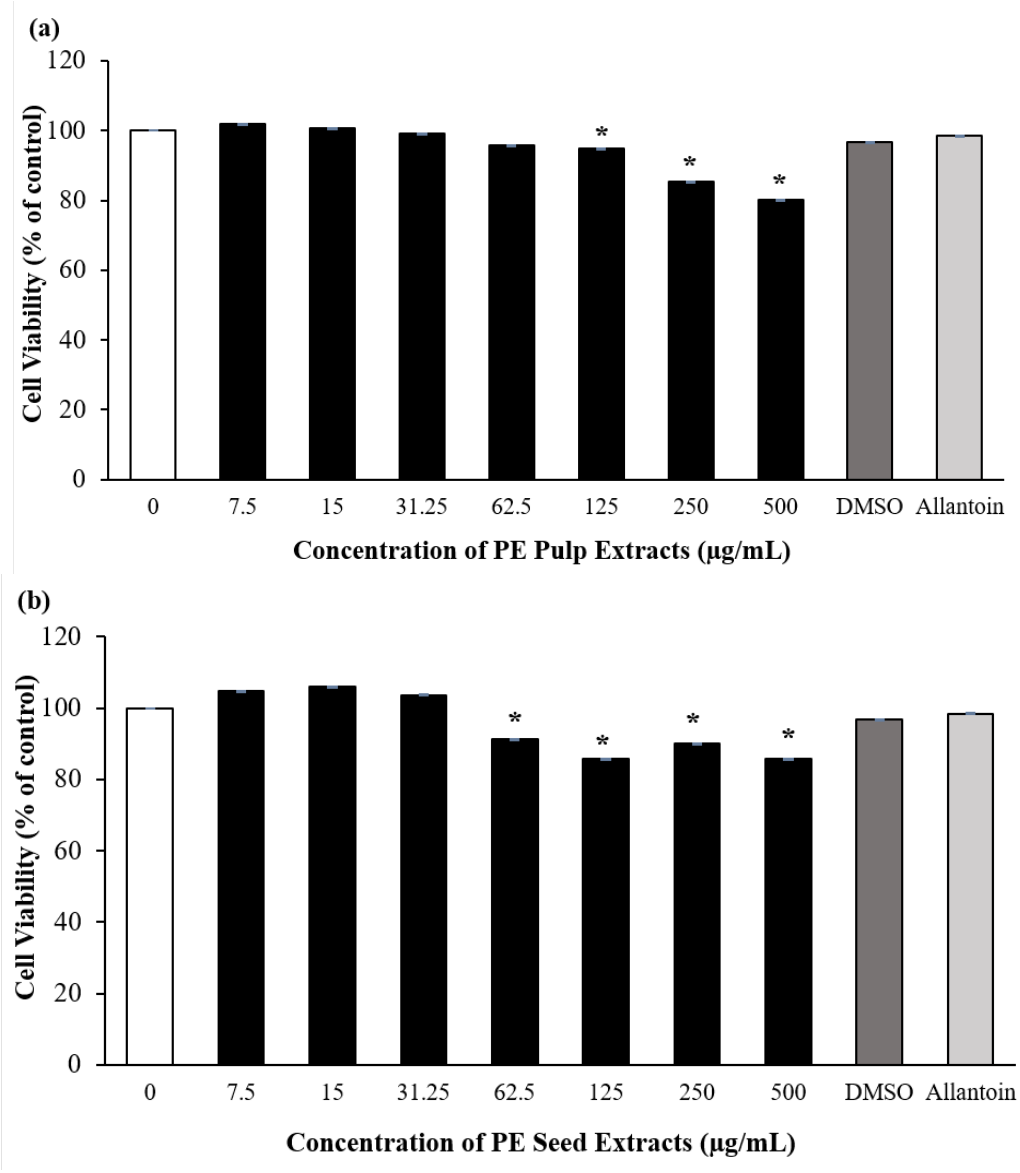

Figure 1. MTT-based cytotoxicity assay. HDF cells at 2.2 × 104 cells/well in 200 µl growth medium were incubated with PE lipid (a) pulp and (b) seed extracts (7.5-500 µg/mL) for 24 h. DMSO at 0.05% served as a vehicle control while 125 µg/mL of allantoin served as a positive control. The data were shown as mean ± SD (n = 6). A significant difference from 0 µg/mL (control) was indicated by *p<0.05

Effects of *L. garciae* Lipid Extracts on HDF Cells' Chemotactic Motility

The transwell migration assay demonstrated that treatment with 31.25 µg/mL of PE lipid pulp and seed extracts significantly enhanced the chemotactic motility of HDF cells compared to negative controls (SFM and growth medium) (Figure 4). The concentration of 31.25 µg/mL of the PE lipid pulp and seed extracts was chosen based on its effectiveness in improving cell migration as observed in the wound scratch assay. The results suggested that PE lipid pulp and seed extracts possess the ability to induce chemotactic response thereby increasing the motility of HDF cells.

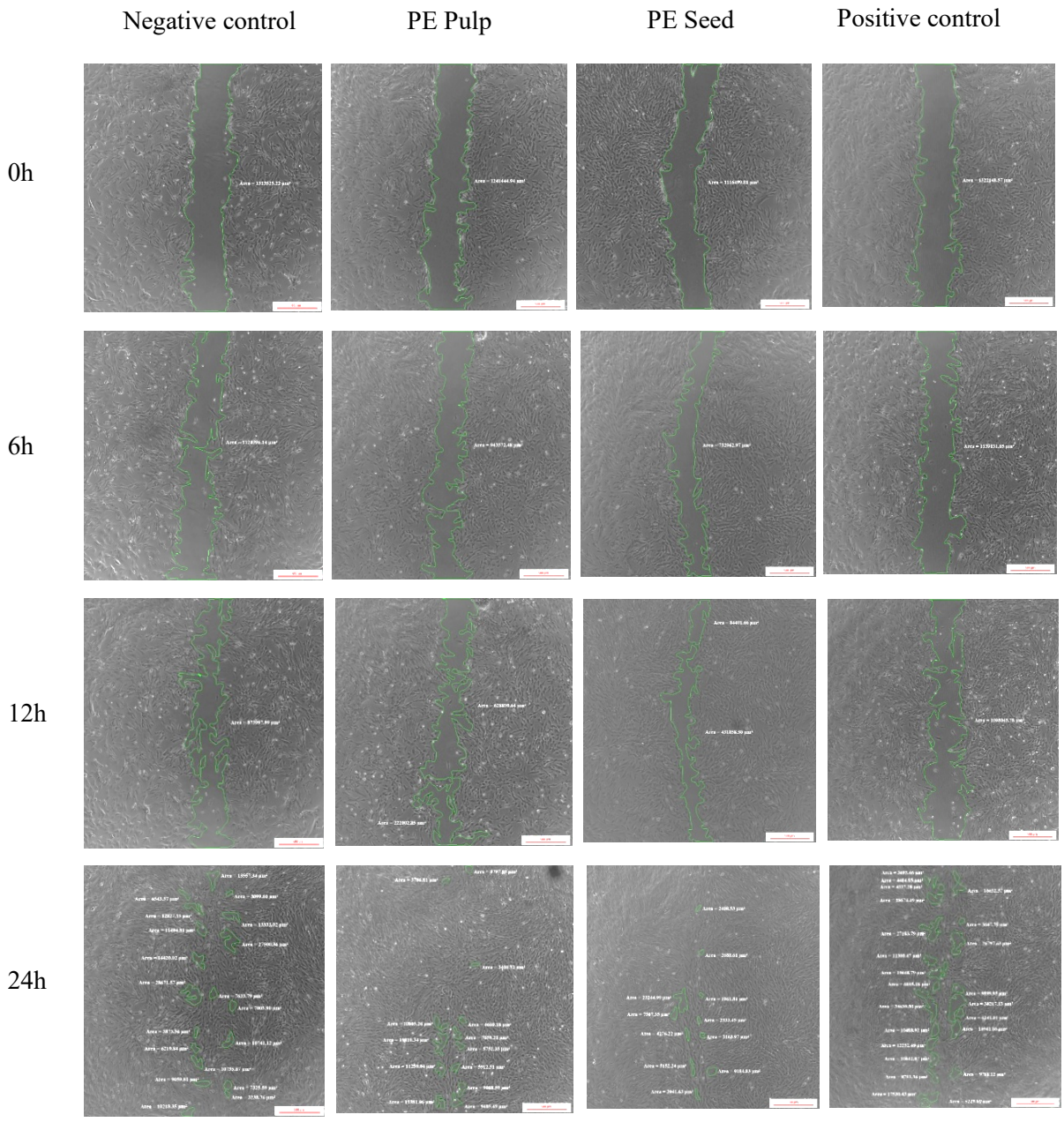

Figure 2. Images of migration of HDF cells using wound scratch assay. The cells treated with 31.25 µg/mL PE lipid pulp and seed extracts was improved after 24h as compared with the negative control. The negative control was the untreated cells supplemented with serum-reduced medium while the positive control was the cells treated with 125 µg/mL allantoin

Effects of *L. garciae* Lipid Extracts on the Collagen Type I Synthesis

Collagen type I is vital for wound healing process due to it attracts fibroblasts and enables stimulation of new collagen deposition at lesion site. Moreover, optimal deposition of collagen is essential for the formation of connective tissue and the prevention of scarring. HDF cells with the treatment of 31.25 µg/mL PE lipid pulp extract significantly increased collagen type I synthesis ($2.03 \times 10^5 \pm 0.37$ pg/mL) compared to the negative control ($1.17 \times 10^5 \pm 0.02$ pg/mL) (Figure 5a). Incubation of HDF cells with 7.5 µg/mL of PE lipid seed extract also led to a notable increment in the synthesis of collagen type I in comparison with negative control (Figure 5b). Interestingly, HDF cells treated with the positive control, allantoin, did not improve collagen type I synthesis.

Effects of *L. garciae* Lipid Extracts on the Secretion of Epidermal Growth Factor (EGF)

EGF acts as an important component for stimulating fibroblast proliferation and migration during healing of wound. It stimulates collagen synthesis and regeneration of skin through the keratinocyte-fibroblast interactions (Kim *et al.*, 2015). There were improvements in EGF secretion levels for HDF cells treated with all the concentrations of PE lipid pulp extracts (Figure 6). Treatment of HDF cells with 7.5, 15 and 31.25 µg/mL of PE lipid pulp extracts resulted in increases in EGF secretion by 8.77%, 24.00% and 15.38% respectively, compared to the negative control. However, PE lipid seed extracts did not show improvement in EGF secretion. Similarly, the positive control, allantoin, also showed no effect on EGF secretion.

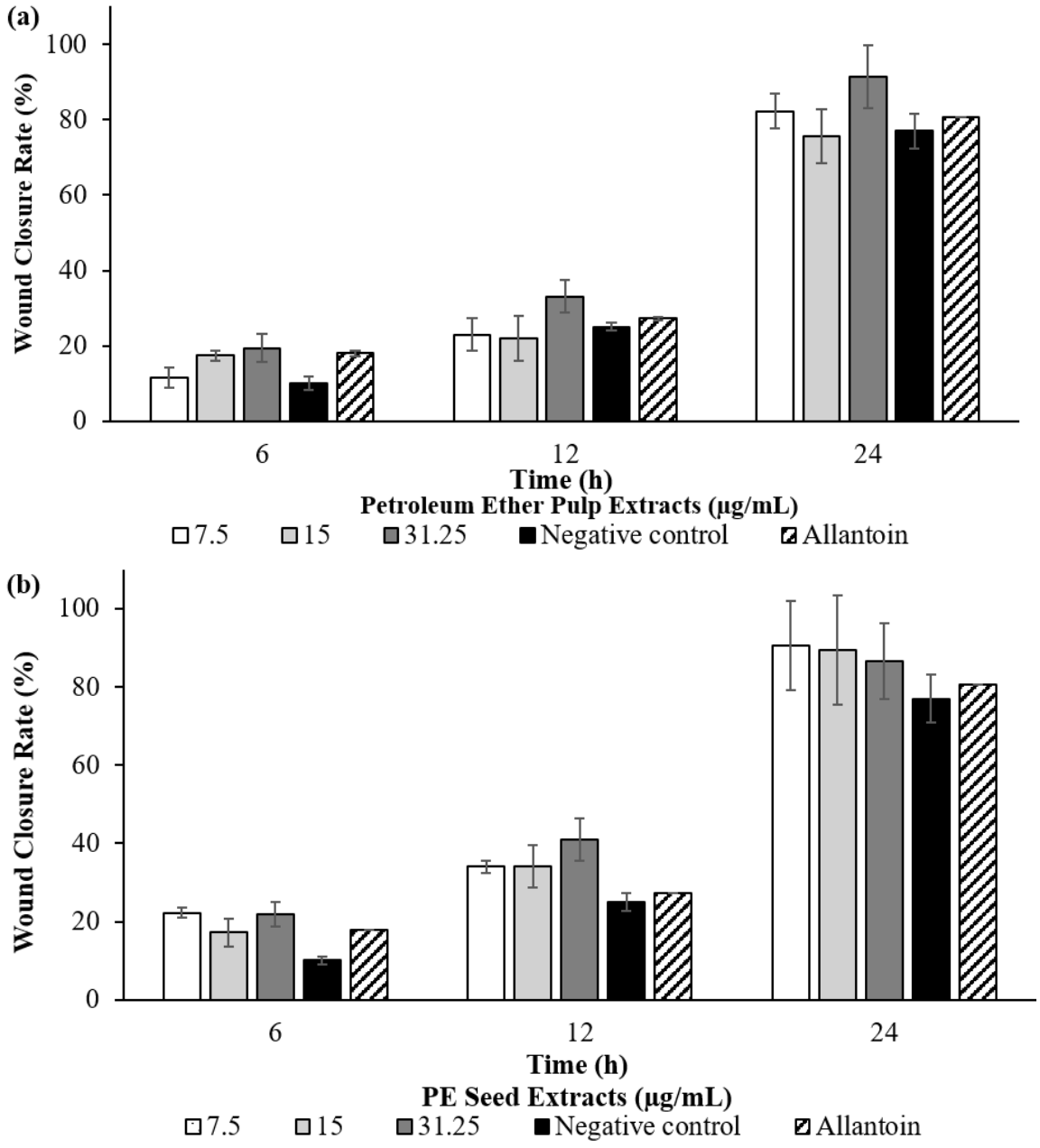

Figure 3. HDF cell migration assay. The migration of HDF cells was measured using the wound scratch assay. Enhanced migration was noted in the cells treated with PE lipid (a) pulp (7.5 - 31.25 µg/mL) and (b) seed (7.5 – 31.25 µg/mL) extracts as compared with the negative control (0 µg/mL) for 24 h. The data were expressed as mean ± SD (n = 3). A significant difference from negative control was indicated by *$p<0.05$

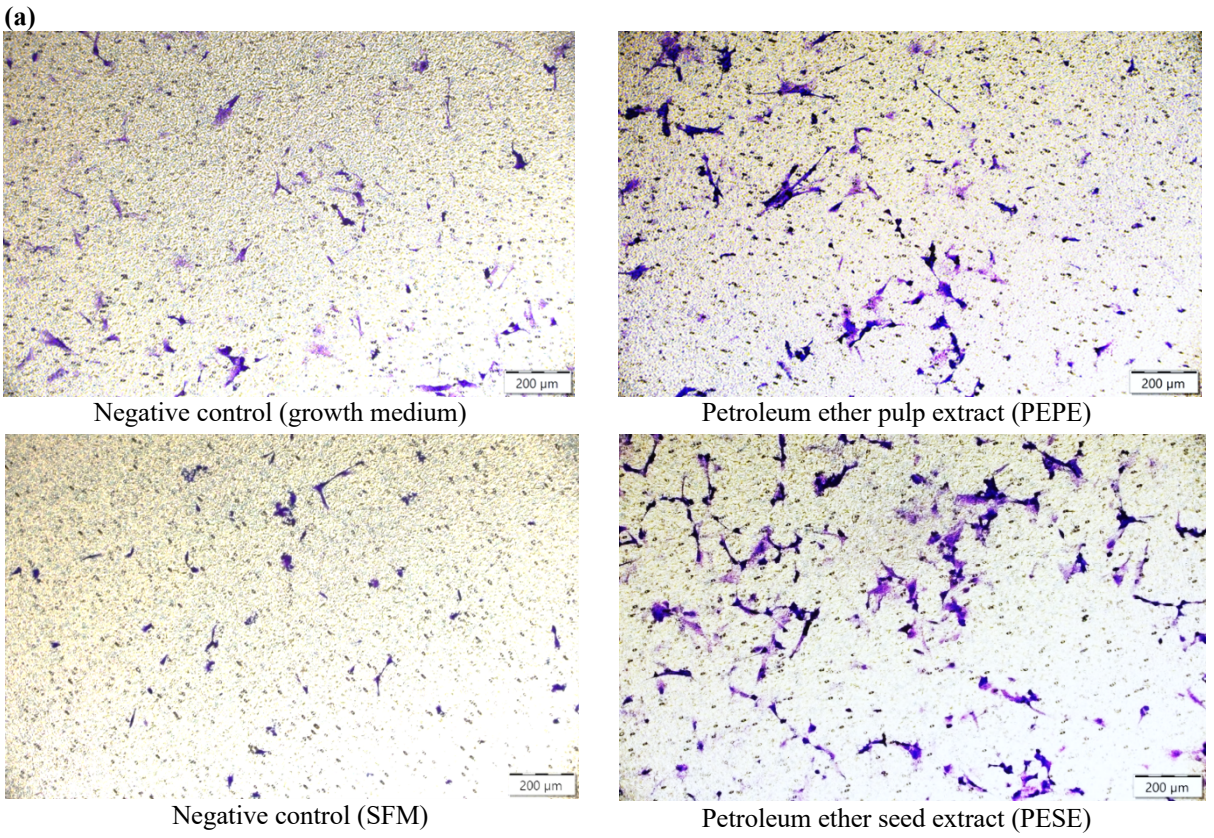

Figure 4. Chemotactic motility of HDF cells detected by transwell assay. (a) The migrated cells for negative control in growth medium, negative control with SFM, cells treated with 31.25 µg/mL of PE lipid pulp and seed extracts. (b) The number of migrated cells on the lower chamber membrane were stained and counted. The data were expressed as mean ± SD (n = 3). Statistical significant differences between PE lipid pulp and seed extracts and negative controls (SFM and growth medium) were represented by (*) and (#) respectively ($p<0.05$)

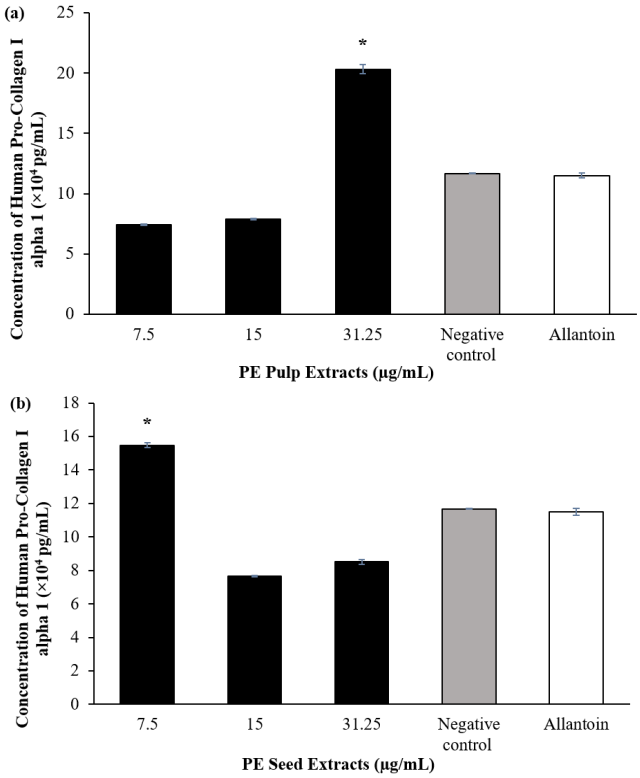

Figure 5. The influence of PE lipid (a) pulp and (b) seed extracts on the expression of collagen type I by wounded HDF cells. Data were expressed as mean ± SD (n = 3). Statistically significant differences between PE lipid pulp and seed extracts and negative control were represented by (*p<0.05)

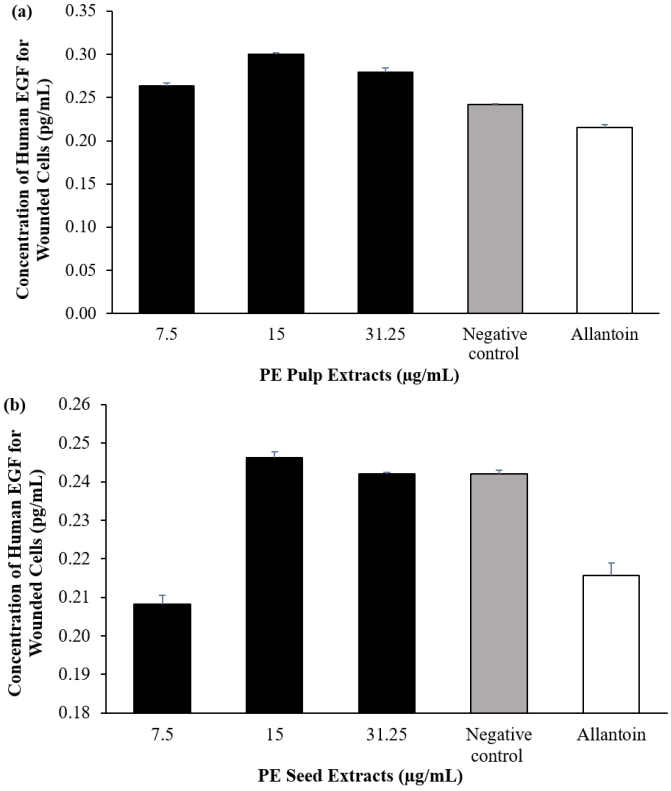

Figure 6. The influence of PE lipid (a) pulp and (b) seed extracts on the secretion of EGF by wounded fibroblasts. The data were presented as mean ± SD (n = 3)

DISCUSSION

Wound healing involves complex biological events categorised into four primary phases: haemostasis, inflammation, proliferation, and remodelling. A large number of growth factors (EGF, collagen and VEGF) and different cell types (platelets, neutrophils, macrophages, lymphocytes, fibroblasts, keratinocytes, and endothelial cells) play distinct roles at different stages for the process of wound repair (Broughton *et al.*, 2006; Guo & DiPietro, 2010). Fibroblasts act as the main cell types present in the skin layer (dermis). Their crucial roles in wound healing include producing several regulatory molecules and interacting with other immune cells that included in wound repair process. Fibroblasts serve as source of new extracellular matrix (ECM) and collagen structures that support other cells associated with effective wound healing, thereby restoring the skin function (Singer & Clark, 1999; Kim *et al.*, 2007).

Fibroblasts migration is important in healing the wound, initiating proliferative phase of wound repair process (Tschumperlin, 2013; Addis *et al.*, 2020). It serves as a rate-determining step vital for the granulation tissue formation (Cen *et al.*, 2022) which modulates subsequent phases, for instance, angiogenesis, inflammation, and re-epithelialisation. During tissue remodelling, granulation tissue develops into mature connective tissue with full recovery of function and structure (Häkkinen *et al.*, 2011). Defects in granulation tissue generation can result in prolonged wound healing and chronic wounds.

Litsea garciae PE lipid pulp and seed extracts were found to enhance the fibroblast migration rate potentially attributed to their fatty acids content and phytochemicals. de Oliveira *et al.* (2013) revealed that improved re-epithelialisation was due to presence of phytochemicals and fatty acids, for instance, oleic, linolenic and linoleic acids. Guidoni *et al.* (2019) revealed that these compounds can stimulate fibroblast migratory activity, promoting granulation tissue production and accelerating wound healing. Additionally, Rojo *et al.* (2010) had demonstrated promotion of fibroblasts migration required fatty acids mixture (mixture of linoleic and oleic acids) as treatment with only oleic acid or linoleic acid did not increase cell migration. Hence, it is hypothesised that the combination of oleic and linoleic acids in the *L. garciae* extracts is essential for enhancing fibroblast migration in the present study, where the synergistic effects between oleic and linoleic acids are inferred.

The chemotactic motility or chemotaxis of fibroblasts refers to their migration towards chemoattractant such as plant extracts (Stock & Baker, 2009). This process is crucial in wound repair process, where chemotactic gradients control fibroblast migration and extracellular matrix synthesis (Postlethwaite, 1987). In this study, the PE lipid extracts enhanced the fibroblasts' chemotactic motility compared to the negative controls (SFM and growth medium). Hence, a significant chemotactic effect of *L. garciae* extracts on HDF and enhancement of HDF migration suggest a positive effect of the extracts on improving wound healing. Previous finding reported by Smith *et al.* (2011) found the unsaturated fatty acids (oleic and linoleic acids), improved chemotactic motility of mesenchymal stem cells (MSC). Since MSC could differentiate into different types of cell lineages, for instance, fibroblasts (Caplan, 2005), the enhancement in the fibroblasts' chemotaxis might be contributed by the high proportion of oleic acid and linoleic acid in *L. garciae* extracts.

Moreover, the phenolic compounds such as quercetin, gallic acid, and rutin were responsible for the stimulation of the Wnt/β-catenin signalling pathway and accelerated the motility of fibroblasts (Seo *et al.*, 2016). The activation of the Wnt/β-catenin signalling pathway regenerated the wound healing activities such as the proliferation of cells and tissue remodelling (Choi *et al.*, 2022). The *L. garciae* bark extracts contained phenolic and flavonoid compounds (Raduan *et al.*, 2022), where these compounds exerted antimicrobial, antioxidant and anti-inflammatory properties and initiated the migration of cells (Comino-Sanz *et al.*, 2021; Nguyen & Bhattacharya, 2022). These properties might result in wound healing improvement and skin restoration. Hence, the promotion of fibroblasts' chemotactic motility might be attributed to the presence of quercetin, gallic acid and rutin which plays an essential role in activating the Wnt/β-catenin signalling pathway and ameliorates the wound healing process. In conclusion, the presence of phenolic

and flavonoid compounds and linoleic and oleic acids in the *L. garciae* extracts might ameliorate the migration and chemotactic motility of fibroblasts. Hence, these findings affirmed the capability of *L. garciae* extracts to improve the wound healing process.

The wound healing can be regulated by collagen type I and growth factors, EGF. Fibroblasts play an essential role in all phases of wound repair, where they secrete collagens, cytokines, growth factors and other components of ECM (Schäfer & Werner, 2007). Collagen type I is the dominant protein for skin ECM formation (Tracy *et al.*, 2016) and modulates the wound healing process by controlling cellular mitogenesis, differentiation, proliferation, and migration. The fibroblast proliferation and migration were also mediated by Erk1/2 and p38 MAPK signalling pathways (Ranzato *et al.*, 2008). The deposition of collagens contributes to structural support to the connective tissue, which enhances tensile strength of wound (Rangaraj *et al.*, 2011). Moreover, growth factors trigger the inflammation phase and stimulating cellular proliferation, angiogenesis, and migration (Vaidyanathan, 2021). EGF induces fibroblast proliferation and migration which results in wound contraction and reduces the wound site area (Bodnar, 2013).

In the current study, the PE lipid pulp and seed extracts significantly increased the collagen type I synthesis. The upregulation of expression of collagen type I may initiate fibroblast migration (Bolla *et al.*, 2019). The promotion of collagen type I generation was shown to be enhanced by an extracellular signal-regulated kinase (Erk) 1/2 and p38 mitogen-activated protein kinase (MAPK) (Park *et al*., 2020). Furthermore, Bolla *et al*. (2019) stated that the upregulation in the expression of collagen type I was assumed to initiate the migration of fibroblasts. This was attributed to the presence of phytochemicals in the plant extracts such as flavonoids and triterpenoids, which possessed antioxidant properties and ameliorated the wound healing process (James & Friday, 2010). Moreover, Cardoso *et al.* (2004) showed that linoleic acid promoted a greater nitric oxide (NO) production at the wound site of mice, which resulting in improved synthesis of collagen by fibroblasts (Frank *et al*., 2002). Therefore, the increment in the expression of collagen type I in this study might be attributed to the phytochemical constituents and linoleic acid that were present in the *L. garciae* extracts.

In addition, all the concentrations of PE lipid pulp extracts demonstrated enhancement in EGF secretion. The enhancement in the expression of EGF level increased fibroblast proliferation and migration, induced neovascularisation, accelerated reepithelialisation rate of wound, and ultimately expediting wound repair process (Li *et al*., 2019). Furthermore, Ili and Sari (2023) demonstrated that egg yolk oil improved the wound repair through elevating EGF and VEGF secretion at the lesion site. This was because fatty acids composition in egg yolk oil which were predominated by the palmitic, linoleic and oleic acids. Therefore, improvement in the expression of the EGF level in the current study might be attributed to the presence of oleic, linoleic and palmitic acids in *L. garciae* extracts. In short, there was no significant difference between the expression levels of collagen type I and EGF between the pulp and seed extracts. This was due to the presence of oleic, linoleic and palmitic acids in both pulp and seed extracts that could promote the synthesis of collagen type I and EGF.

CONCLUSION

L. garciae lipid pulp and seed extracts increased the collagen type I secretion levels and EGF and induced chemotactic responses in HDF cells. These characteristics improved the HDF cells migration and promoted the wound healing process. The findings contribute preliminary data on the potential of *L. garciae* lipid extracts to promote wound healing process. Due to the limitation of *in vitro* wound healing study, an *in vivo* wound healing activity of the *L. garciae* extracts can be further evaluated to confirm the safety and effectiveness of the extracts that may aid in wound healing process.

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