**In vitro** evaluation of the effects of *Inula viscosa*’s different extracts on wound healing and oxidative stress in mouse L929 fibroblast cell line

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Research Article

**Keywords:** Medicinal plants, extraction, scratch assay, cell proliferation, oxidative status

**Posted Date:** February 2nd, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-2531325/v1

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Abstract

This study evaluated the effects of extracts prepared using two different methods (decoction extraction and, Soxhlet ethyl acetate/hexane extraction) from *Inula viscosa* on H$_2$O$_2$-induced oxidative stress and wound healing model in mouse L929 fibroblast cells. The cytotoxic effect started to disappear statistically (p < 0.05) at concentrations of Soxhlet ethyl acetate/hexane extracts (SoxEHEs) in and below 0.1 mg/mL, while the same effect was observed at concentrations of decoction extracts (DEs) in and below 0.2 mg/mL. Therefore, 0.2 and 0.02 mg/mL concentrations of DE, and 0.1 and 0.01 mg/mL concentrations of SoxEHEs were used. While cell migration was positively affected in all concentrations of the research, statistically significant results (p < 0.05) were obtained from 0.2 mg/mL of DE and 0.1 mg/mL of SoxEHE extracts. Malondialdehyde (MDA) levels were found to be statistically (p < 0.05) decreased, but COL1A1 levels were higher in cell lines treated with oxidative stress + extract than in the cell line treated only with H$_2$O$_2$, and reduced glutathione (GSH) levels were higher in cell lines only treated only with extract than in oxidative stress-induced cell lines. Consequently, it has been observed that the extracts have positive effects on migration and oxidative stress. Therefore, *I. viscosa* may serve as a new therapeutic agent for wound healing.

Introduction

A wound is a deterioration of the cellular, anatomical, and functional continuity of living tissue due to a physical, chemical, thermal, microbial, or immunological effect on living tissue. In contrast, wound healing is a biological process consisting of various biochemical and cellular pathways to restore the structural and functional conditions of injured tissues and improve physiological conditions [1]. Wound healing is a continuous and overlapping process that is staged as hemostasis (accepted within the inflammation stage in the three-stage concept), inflammation, new tissue formation, and remodeling of tissue [2]. Redox signalling and oxidative stress play important roles in regulating normal wound healing and, contribute to specific stages [3]. The formation of reactive oxygen species (ROS) at low concentrations is necessary not only for the fight against invasive microorganisms but also for the signaling of cells during the wound healing process [4]. However, even if ROS production is essential for initiating wound repair, excessive ROS formation and uncontrolled oxidative stress in wound healing contribute to persistent and uncontrolled inflammation, which plays an important role in the pathogenesis of chronic non-healing wounds [1]. Problems related to wound healing can occur in the form of swift recovery (such as hypertrophy and keloid scars) [2], or these problems may be seen as delayed wound healing in especially diabetics or elderly people [5]. The healing time of chronic non-healing wounds lasts on average 12–13 months and repeats in 60–70% of patients. If they are not treated, it causes death in addition to loss of function and a decrease in quality of life [6]. Although there are various alternatives for the treatment of wounds from topical applications (hydrogel, povidone-iodine solution, cadexomer iodine, etc.) to advanced treatment methods such as growth factors, extracellular matrix and, negative pressure wound treatment [6], these methods have sometimes remained incomplete and impractical. Therefore,
the scientific world has constantly been searching for methods that are effective both in practice and cost-wise.

Various herbal products have been used in wound treatment over the years, and these phytochemical compounds have been reported to fight infections, promote blood clotting, and accelerate the healing process. When these wound-healing plants are investigated, it has been claimed that several of them have the potential to increase wound healing owing to their high antioxidant properties [1]. The genus Inula, belonging to the Asteraceae family, has more than hundred species, found mainly in Africa, Asia (20 species are also found in China), and Europe, especially in the Mediterranean region [7]. The genus Inula includes several species with medicinal significance established by several applications in traditional medicine, biological activities of their extracts, and pure secondary metabolites [8]. *Inula viscosa* [*Dittrichia viscosa* (L.) Greuter] is used in folk medicine in the Mediterranean region for its anti-inflamatory, antipyretic, antiseptic, antiphlogistic, and balsamic activities, as well as for the treatment of lung and gastroduodenal disorders [9]. Antioxidant, antibacterial, antifungal, hypoglycemic, hypolipidemic, anticancer, antiparasitic and phytotoxic effects have also been reported for *I. viscosa* extracts, and it has been argued that these effects originate from sesquiterpenoids, triterpenoids, and flavonoids [10].

A literature review has shown some reports of extracts and purified metabolites obtained from *I. viscosa*; however, only a morphological study has been conducted in rats on the wound healing process. Khalil et al. (2007) [11] reported that only 10% aqueous extract of *I. viscosa* healed both the morphological and histological features of wounds. However, no physiopathological studies have shown the effects of different types of extracts obtained from *I. viscosa* on wound healing.

As a result, we examined the *in vitro* effects of different extracts of the *I. viscosa* plant on wound healing in L929 cells with hydrogen peroxide-induced oxidative stress and levels of oxidative stress parameters such as malondialdehyde (MDA) and reduced glutathione (GSH), and COL1A1 gene expression, which have an effect on healing in this process.

**Materials And Methods**

**Preparation and extraction of plant material**

After the plants collected from Manisa/Akhisar was diagnosed by Asst. Prof. Dr. Mustafa Kemal Altunoglu (Kafkas University, Faculty of Sciences and Letter, Department of Biology, Kars), the aerial parts were dried in a dry place for two weeks. Dried plants were powdered using an electric blender.

**Ethyl Acetate/hexane Extraction**

The plant extract was obtained using a Soxhlet extraction system. Forty (40) grams of herb were weighed, wrapped in filter paper, and placed in a soxhlet apparatus. Ethyl acetate and n-hexane (3:1 v/v)
were used as solvents. The temperature of the system reached the optimum level, and the extraction process was terminated after 3 h. The remaining solvent in the extract was vaporized at 60ºC in a rotary evaporator until it was completely removed. Before testing, dilutions of the ethyl acetate/hexane extract (SoxEHE) (10, 1, 0.1, 0.01, and 0.001 mg/mL) were prepared.

**Extraction by method of decoction.**

In this study, a 2% extract of the plant material was prepared. Twenty grams of the plant were weighed and transferred to a teapot. After adding 1 liter of cold distilled water, it was left to boil. After boiling, it was waited for 10 minutes and allowed to cool to room temperature. It was filtered through filter paper to avoid residue. Dilutions (20, 2, 0.2, 0.02, and 0.002 mg/ml) were prepared from decoction extract (DE) immediately prior to testing.

**L929 Fibroblast Cell Proliferation And Viability Analysis**

The L929 (mouse fibroblast cell, ATCC CCL-1) cell line was purchased from American Type Culture Collection (ATCC, USA) and was used for the experiments. In the liquid nitrogen tank, the cell lines on the cryotube were removed from the tank and kept in a water bath for a short period to dissolve at 37°C. The dissolved cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum in 75 cm² plastic flasks. After 48 h, L929 cells were counted in DMEM containing 10% fetal bovine serum (FBS) at a density of 2x10⁵ cells/well, and plated in a well plate with 96 wells, and incubated at 37°C in a humid atmosphere containing 5% CO₂. For 24 h, the effects of DE concentrations (20, 2, 0.2, 0.02, and 0.002 mg/mL) and SoxEHE concentrations (10, 1, 0.1, 0.01, and 0.001 mg/mL) in L929 cells were investigated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method to determine toxic doses of decoction extracts (dissolved in pure water) and ethyl acetate (dissolved in 0.01% DMSO and applied to cells). After the appropriate doses were determined, the cells were planted again in a well plate with 96 wells. For 24 h, the cells were exposed to 2 mg/mL and 0.2 mg/mL decoction and 0.01 mg/mL and 0.001 mg/mL ethyl acetate extracts at different concentrations, and then 3 hours later H₂O₂ (0.75 mM) was added to the media. After the MTT method was applied to the cells, at 24th hours, the absorbance was read in each well at 620 nm using a microtiter plate ELISA reader (Epoch Microplate Spectrophotometer, BioTek, USA). The assays were performed a minimum of three times for each repetition. The survival rates of L929 cells were analyzed by comparing them with those of the control wells.

**Migration Test**

The migration of L929 fibroblast cells was examined using the wound healing method. Briefly, L929 cells (2x10⁵ cells/mL) in DMEM containing 10% FBS were seeded into each well of a 24-well plate and incubated at 37°C and 5% CO₂. When the cells formed a complete monolayer in the wells, a scratch was created horizontally in each well with a sterile pipette tip. Cellular debris was removed by washing with
phosphate buffered saline (PBS) and replaced with 2 mL of fresh medium without the addition of test samples. On day 0, images were taken using the Invitrogen Inverted Microscope, and the wells were photographed at 0, 12 and 24 h by keeping them in the incubator.

**Determination Of Oxidant/antioxidant Parameters (MDA, GSH)**

L929 cells (2x10^5 cells/mL) were seeded in each well of 6-well plates (separately for the analysis of MDA and GSH) and incubated in a humid environment containing 5% CO_2 at 37°C. Using the scraping method, cells were removed from the 6-well plates and stored at -80°C. Approximately 100 mg of cell lysate from each group was homogenized with Tissue Lyser on ice in a specific homogenate buffer. The samples were then centrifuged. For biochemical studies, while MDA levels in supernatants were measured colorimetrically using the methods reported by Yoshioka et al. (1979) [14], GSH levels were measured colorimetrically as described by Beutler et al. (1963) [15].

**Gene Expression Analysis By RT-PCR**

Gene expression analysis by RT-PCR

Total RNA was isolated from exponentially growing cells using TRIzol Reagent (Sigma). The total RNA was treated with RQ1 DNAse I (Promega). Reverse transcription (RT) was performed according to the manufacturer’s instructions (Fermentas) using 1 unit of MMLV reverse transcriptase with 5 µg of total RNA. The COL1A1 protein-coding cDNA region was amplified using the F and R primers (Table 1).

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer List</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL1A1F</td>
<td>GGCACCTCCGGACCTCAAG</td>
<td>64</td>
</tr>
<tr>
<td>COL1A1R</td>
<td>CGGTCACCGTTCTTGCCAG</td>
<td>62</td>
</tr>
</tbody>
</table>

**Statistical analysis**

All data for statistical analysis were calculated using Microsoft Excel, and the results obtained were shown as the mean ± standard deviation. For data analysis, one-way variance analysis (ANOVA) was performed using the IBM 25.00 SPSS statistical program, followed by Tukey’s test. p < 0.05 was considered as important.

**Results**

**Proliferation of L929 and viability analysis**
In this study, when the effects of decoction and ethyl acetate extracts on cell proliferation were evaluated separately, DEs demonstrated cytotoxic effects at doses of 20 mg/mL and 2 mg/mL ($p < 0.05$). At doses of 0.2 mg/mL and below, the cytotoxic effect in healthy fibroblast cells began to disappear (Fig. 1I). The efficacy of 0.2 mg/mL, 0.02 mg/mL and 0.002 mg/mL were statistically similar ($p > 0.05$). Therefore, we decided to use DE at concentrations of 0.2 and 0.02 mg/mL in the later stages of our study. The cytotoxic effect of SoxEHEs on healthy fibroblast cells at doses of 10 mg/mL and 1 mg/mL is shown in Fig. 1I ($p < 0.05$). This cytotoxic effect was observed to be eliminated at 0.1 mg/mL and lower doses. The effects of SoxEHE at doses of 0.1, 0.01, and 0.001 mg/mL were statistically similar ($p > 0.05$). Therefore, SoxEHE was found to be effective at doses of 0.1 mg/mL and 0.01 mg/mL.

**The Effect Of Ho On L929 Cells**

When Fig. 1II was examined, it was observed that $\text{H}_2\text{O}_2$ at a dose of 0.75 mM significantly reduced cell viability compared to the control group ($p < 0.05$). The decoction extract significantly improved cell viability by protecting $\text{H}_2\text{O}_2$ damage, especially at a dose of 0.2 mg/mL ($p < 0.05$). Ethyl acetate/hexane extract was able to maintain cell viability significantly against oxidative damage induced by $\text{H}_2\text{O}_2$ at a dose of 0.1 mg/mL ($p < 0.05$). Both extracts showed lower effectiveness at decreasing the doses.

**Migration Test Results**

A migration test (wound healing test), a standard *in vitro* method, was performed to examine the effect of the plant extract on the migration of L929 cells to the scratched area, the so-called injured part. According to the results, healthy fibroblast cells migrated to the injured area at the 24th hour in such a way that they showed a tendency to close almost completely (Fig. 2I; A1-C1). In L929 cells treated with only hydrogen peroxide, it was observed that cell migration was prevented, and this was statistically significant ($p < 0.05$) in the measurements made at the 12th and 24th hours (Fig. 2I; A2-C2). $\text{H}_2\text{O}_2$ showed both a cytotoxic effect by creating oxidative damage and caused the wound to not heal by preventing cell migration. At the doses in which DE was applied, cell migration to the injured area increased significantly at both doses. Unlike the MTT results, there was more healthy migration, especially at the dose of 0.02 mg/mL ($p < 0.05$) (Fig. 2II; A2-C2). In groups where SoxEHE was applied, cell migration increased in both doses, while it significantly increased cell migration against $\text{H}_2\text{O}_2$ at a dose of 0.1 mg/mL (Fig. 2III; A2-C2, 3).

**Results Of MDA And GSH**

When the levels of MDA as a signal of lipid peroxidation were examined in the L929 cell line, it was observed that the highest level ($p < 0.05$) was in the oxidative stress-induced cell line compared to other cell lines (Fig. 4). In the cell lines, where the effect of plant extract on oxidative stress was observed, MDA levels were significantly lower ($p < 0.05$) compared to the cell line where only $\text{H}_2\text{O}_2$ was applied, but they...
varied among themselves. The lowest MDA levels in these cell lines (except for the control cell line) were seen in DE (0.02 mg/mL) + H2O2 and SoxEHE (0.1 mg/mL) + H2O2 cell lines. These results indicated that 0.02 mg/mL concentration of DE and 0.1 mg/mL concentration of SoxEHE were effective on lipid peroxidation.

When GSH levels as a tripeptide antioxidant in all cell lines were analyzed, statistically high levels (p < 0.05) were found to be the only ones in the extracted cell lines. This suggested that plant extracts were effective in improving antioxidant capacity (Fig. 4). The GSH levels of the plant extract and H2O2-treated cell lines decreased statistically (p < 0.05) to almost the level of the cell line applied to H2O2 alone (except SoxEHE (0.01 mg/mL) + the H2O2 cell line) compared to only the plant extract applied cell lines. This could be explained by GSH depletion during oxidative stress.

**Mrna Distribution Of Col1a1**

The mRNA expression levels of COL1A1 were investigated using RT-PCR. The increase COL1A1 levels in these cell lines (except for the control cell line) were seen in DE (0.2 mg/mL) + H2O2 and SoxEHE (0.1 mg/mL). These results indicated that 0.2 mg/mL concentration of DE and 0.1 mg/mL concentration of SoxEHE were effective on wound healing (Fig. 5). The level of the GAPDH transcript was the same at all time intervals (Fig. 5). When COL1A1 levels as collagen were analyzed in all cell lines, it was found that high levels were only present in the extracted cell lines, therefore, plant extracts were effective in improving collagen capacity (Fig. 5).

**Discussion**

A large percentage of the human population around the world tends to use herbal medicines because they find them more reliable and easier to access. They also realize synthetic drugs and their side effects. Throughout history, many plant extracts, mixtures, porridge, boiling, and pastes have been used in many countries for the treatment of diseases, cuts, wounds, and burns. Therefore, since ancient times, several herbs and plant-based strategies have been known for their important roles in wound healing and skin regeneration, as well as in their therapeutic application [1].

Scratch testing in fibroblast cell cultures is a method that is widely applied to identify components of wound healing that have been investigated [16]. Oxidative stress originating from hydrogen peroxide is an alternative to assess the antioxidant activity of the extract in cells and to delay stress-induced wound healing [17]. In this study, *I. viscosa* extracts prepared using different methods were used to test the activity of fibroblast scratch wound healing in H2O2-induced L929 cells and the effects of H2O2-induced oxidative stress.

Since cell proliferation and migration are two important characteristics in the tissue formation phase of wound healing, it was important to determine the non-toxic dose of the extracts in the cell line where we investigated the therapeutic effect. Therefore, firstly the effects of the extracts on proliferation and cell
viability of L929 fibroblast cells were indicated. 0.2 mg/mL and decreasing dilutions for DEs, 0.1 mg/mL and decreasing dilutions for SoxEHEs were found to be non-toxic, and it was observed that cell viability was more than 80%.

In a study in which the cell viability of *I. viscosa* extracts obtained by two different methods was examined using the XTT method, it was reported that 50 µg/mL decoction extract produced a cell viability of over 80% in the L929 fibroblast cell line [18]. The results of this study are consistent with those of our study.

At the concentrations tested in our study, 20 mg/mL of DE and 10 mg/mL of SoxEHE were considered to have cytotoxic effects in the L929 fibroblast cell line (Fig. 1I). Therefore, it is possible that extracted or purified phytochemicals obtained from the Inula genus may have antiproliferative properties [9, 19].

Free radicals formed at the basal level during wound healing are necessary for cell signaling and fight against invasive microorganisms. H$_2$O$_2$ ensures that leukocytes are drawn into the wound area as chemoattractant after wound formation and also participate in later stages of healing [20]. When an excessive amount of free radicals is produced, a condition called oxidative stress occurs, which disrupts the structural integrity of the cell by damaging DNA, carbohydrates, proteins, and especially lipid structures in membranes. Therefore, wound healing is delayed, or the wound cannot heal [21].

Oxidative stress originating from hydrogen peroxide is an alternative method for evaluating the antioxidant activity of extracts in cells [22]. The determination of MDA, a method used to measure oxidative stress, is based on the reaction of malondialdehyde formed by the peroxidation of polyunsaturated fatty acids with thiobarbituric acid [14]. In the present study, MDA levels were higher in all groups compared to the control, but the highest level was only in the H$_2$O$_2$ treated cell line without statistical difference. This indicate that H$_2$O$_2$ induced oxidative stress in this group. Increased ROS levels can inhibit cell migration and proliferation [23]. The absence of cell migration and proliferation at the 12th and 24th hours in the group treated with H$_2$O$_2$ alone lead to the consideration of oxidative stress and the cytotoxic effect of H$_2$O$_2$. The levels of MDA in the DE (0.2 mg/mL), SoxEHE (0.1 mg/mL) and extract + H$_2$O$_2$ groups were not significantly different. In addition, the fact that cell migration and proliferation were close to the control in groups with oxidative stress and extract, shows that the extracts have a positive effect against oxidative stress.

Antioxidants are protein systems designed to counteract the harmful effects of ROS by providing electrons. Therefore, they prevent the capture of electrons from other important molecules such as DNA, proteins, and lipids [24]. Antioxidants include antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione (GSH) peroxidase, GSH reductase, and other endogenous free radical scavengers such as ascorbic acid, α-tocopherol, and GSH [25]. The H$_2$O$_2$ effect is an interwoven healing process that is controlled by molecular antioxidants such as SOD, GPx, and phospholipid hydroperoxide glutathione peroxidase [17]. Glutathione is an important endogenous antioxidant. It functions as a cellular redox buffer and protects cells against the toxicity of endogenous and exogenous electrophilic compounds [20].
In the study, GSH levels were the highest in the DE (0.2 mg/mL) and SoxEHE (0.1 mg/mL) groups compared to other groups. *In vitro* experimental studies have shown that the extract or purified phytochemical compounds obtained from *I. viscosa* have antioxidant activity [26–28]. Although there were no statistical differences between the extract + H$_2$O$_2$ groups in terms of GSH levels, there is a statistically decrease (p < 0.05) in GSH levels compared to the DE (0.2 mg/mL) and SoxEHE (0.1 mg/mL) groups. The fact that the MDA levels were lower than those the H$_2$O$_2$ group suggests that GSH may have been depleted during migration. The functional importance of antioxidants such as glutathione, ubiquinones, uric acid, lipoic acid, vitamins E and C (ascorbic acid), carotenoids, and phenolic compounds in the wound repair process is suggested by their depletion in healing skin wounds [20]. In a study in which the L929 fibroblast cell line was treated with the GSH blocker, buthionine sulfoximine, cell death occurred following GSH depletion and GSH protected the cells from death [29]. In another study, wounds were reported to heal faster when GSH was applied topically to wounds in diabetic rats. These studies have shown that glutathione is beneficial for wound repair [30].

Fibroblasts, the main cell type in the dermis, are responsible for the production and remodeling of the extracellular matrix during wound healing. Collagen, type III, alpha 1 (COL3A1) and Collagen, type I, alpha 1 (COL1A1) are known to be major extracellular matrix (ECM) components of skin dermal tissues [31]. Beare et al. (2003) [32] observed that the wounds of mice with a mutant collagen type-I gene (COL1A1$^{r/r}$) healed more slowly than those of wild-types mice. Hashimoto et al., (2020) [33] reported that the COL3A1 gene was down-regulated on all surfaces throughout the cell culture. No significant difference in COL1A1 gene expressions was observed on the three surfaces. In the present study, we investigated the effect of this collagen (COL1A1) on wound healing. The increase COL1A1 levels in these cell lines (except for the control cell line) were seen in DE (0.2 mg/mL) + SoxEHE (0.1 mg/mL). These results indicated that 0.2 mg/mL concentration of DE and 0.1 mg/mL concentration of SoxEHE were effective on wound healing (Fig. 5). The level of the GAPDH transcript was the same at all time intervals (Fig. 5). When COL1A1 levels as collagen were analyzed in all cell lines, it was found that high levels were only present in the extracted cell lines, therefore, plant extracts were effective in improving collagen capacity (Fig. 5). In another study, it was reported that COL1A1 and COL3A1 levels increased in parallel with the formation of the fibroblast cell layer [34].

**Conclusions**

Different extraction methods can unearth various phytochemicals in plants. Decoction extraction is less costly than Soxhlet extraction (in terms of both chemicals and equipment). In our study, extracts obtained from *I. viscosa* plant were found to have a positive effect on both migration and oxidative stress parameters in the L929 cell line where oxidative stress was formed. In this context, *I. viscosa* may be a potential agent for the treatment of wounds. However, this needs to be examined in more detail in preclinical and clinical studies.

**Declarations**
Author Contribution  A Harmankaya, Conceptualization; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. Data curation; Resources. İ Çınar, Conceptualization; Formal analysis; Investigation; Methodology; Resources. M Yayla, Conceptualization; Formal analysis; Investigation; Methodology; Resources. S Harmankaya, Investigation; Resources M Beytur, Investigation; Resources. C Öziç, Investigation; Methodology; Resources; Writing-review & editing.

Funding The authors received no specific funding for this work

Data availability Data is available upon reasonable request to the author.

Conflict of interest The authors have no personal, financial, or institutional interest in any of the methods, materials, or devices described in this article. The authors declare no conflicts of interests.

References


Figures
Figure 1

Determination of proliferative and cytotoxic doses of plant extracts (I) and indicating the protective effects of plant extracts against H$_2$O$_2$-induced damage (II) on L929 cells (if the letters in the columns are the same, the difference between them is statistically insignificant, p>0.05 ns: the groups included in the line are meaningless with each other, H$_2$O$_2$: Hydrogen peroxide, DE: Decoction extract, SoxEHE: Soxhlet ethyl acetate/hexane extract).
Illustrating of the cell migration under a scratch assay (wound healing method), I) the migration of healthy cells (A1: 0th time, B1: 12th hour, C1: 24th hour) and the effect of H$_2$O$_2$ application on the cell migration (A2: 0th time, B2: 12th hour, C2: 24th hour), II) the effect of DEs (A1: 0.02 mg/mL; 0th time, B1: 0.02 mg/mL; 12th hour, C1: 0.02 mg/mL; 24th hour, A2: 0.2 mg/mL; 0th time, B2: 0.2 mg/mL; 12th hour, C2: 0.2 mg/mL; 24th hour) and III) the effect of SoxEHEs (A1: 0.01 mg/mL; 0th time, B1: 0.01 mg/mL; 12th hour, C1: 0.01 mg/mL; 24th hour, A2: 0.1 mg/mL; 0th time, B2: 0.1 mg/mL; 12th hour, C2: 0.1 mg/mL; 24th hour) on cell migration against H$_2$O$_2$-induced damage.

Figure 2
Figure 3

Demonstration of the effect of plant extracts on cell migration in an *in vitro* scratch assay (wound healing method) against damage induced by H$_2$O$_2$ in L929 cells as % (If the letters in the columns are the same, the difference between them is statistically meaningless p>0.05, H$_2$O$_2$: Hydrogen peroxide, DE: Decoction extract, SoxEHE: Soxhlet ethyl acetate/hexane extract).
Effect of plant extracts on MDA and GSH levels in an *in vitro* wound healing model against damage caused by \( \text{H}_2\text{O}_2 \) in L929 cells. (If the letters in the columns are the same, the difference between them is statistically insignificant \( p > 0.05 \), DE: Decoction extract, SoxEHE: Soxhlet ethyl acetate/hexane extract).

**Figure 4**
Figure 5

mRNA distribution of COL1A1.