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Assessment of the healing properties of *Cichorium intybus* L. extracts on wounds

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ABSTRACT

Background: Throughout the course of an individual's lifespan, they may come across a diverse range of minor or significant incidents that have the potential to result in the development of scars on any region of their physique. These scars may also be a consequence of the body's innate responses to different stimuli. Various medicinal drugs and methods have been used to treat scars. Herbal remedies have long been popular. *Cichorium intybus* L., or chicory, has been used for centuries to treat numerous illnesses and deformities.

Purpose: The primary objective and novelty of this research endeavor involve the investigation of potential therapeutic attributes inherent in extracts derived from different components of *C. intybus* (common chicory), encompassing the root, stem, flower, and whole herba. This will be accomplished through the utilization of *in vitro* scratch tests and *in vitro* cell migration assays. Additionally, the ash acquired from cremation, a method routinely employed by the general population, was also investigated.

Study design: The focus of this investigation was to evaluate the wound healing effects of these extracts. The research encompassed a comprehensive examination of several components of the plant in order to ascertain the extracts that exhibited the highest efficacy.

Methods: The study utilized the following approaches respectively to investigate the plant's wound healing abilities: collection and identification of plants, preparation of extracts, determination of phenolic compounds by HPLC-PDA, *in vitro* cell culture studies, cell viability analysis, wound healing analysis, statistical analysis.

Results: The findings indicated that the stem extracts of *C. intybus*, with the exception of the hexane extract (Sample 1), demonstrated notable wound healing properties. According to the chromatography results the main component of extract is chlorogenic acid. The aforementioned findings offer significant contributions to the potential directions for future research within this particular sector.

Conclusion: The evidence suggests that extracts from *C. intybus* have wound healing properties, highlighting its significance in traditional medicine and pharmaceutical research. Further investigation into active chemicals and mechanisms contributing to its wound healing properties is needed, potentially leading to advancements in wound healing therapies.

Introduction

Throughout human history, the use of medicinal plants has held a significant place in various cultures and continues to be a frequently used source of medicine today (Dar et al., 2017). *Cichorium intybus* is a member of the Asteraceae family and is a fragrant perennial plant that produces blue and white flowers, reaching heights of up to one meter (Ramakrishna and Ravishankar, 2011). This plant is known for its high stress resistance and can be found in the Van region of Turkey (38° 39′ 24.3936′′ N and 42° 48′ 19.7316′′ E) in both wheat fields and

Abbreviations: ANOVA, Analysis of Variance; DCM, Dichloromethane; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, Dimethyl Sulfoxide; EDTA, Ethylenediaminetetraacetic acid; ELISA, Enzyme-Linked Immunosorbent Assay; FBS, Fetal Bovine Serum; L929, Mouse fibroblast cell line; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, yellow tetrazolium salt; NC, Negative control; PBS, Phosphate-buffered saline; SPSS, Statistical Package for the Social Sciences.

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Table 1
HPLC gradient pump program.

Steps	Flow rate (mL/min)	Time (min)	% Mobile Phase B (Acetonitrile)	% Mobile Phase A (% 0.1 formic acid/ Water)
Step 1	1.00	0.01	5	95
Step 2	1.00	7	9.5	90.5
Step 3	1.00	20	17	83
Step 4	1.00	35	40	60
Step 5	1.00	40	0	100
Step 6	1.00	40.01	Stop	

non-agricultural lands. Ancient Egypt was one of the first civilizations to use and cultivate *C. intybus* for medicinal purposes (Street et al., 2013). This plant has also been widely used for its therapeutic properties in Europe, Asia, and North Africa (López-Muñoz et al., 2006; Süntar et al., 2012). Chicory is a renowned medicinal plant within the framework of traditional Persian Medicine and is particularly advocated for the treatment of hepatic conditions (Bakhshi Jouybari et al., 2018; Sadati et al., 2016).

C. intybus has various uses beyond its medicinal properties. During the wheat harvesting season, this plant can be harvested with wheat and used as hay and animal feed, making it an important resource in livestock breeding. The roots of this plant, which have been used in traditional medicine, have been found to have anti-malarial, anti-ulcerogenic, analgesic, anti-inflammatory, and anti-carcinogenic properties (Bischoff et al., 2004; Cavin et al., 2005; Conforti et al., 2008; Gürbüz et al., 2003; Petrovic et al., 2004; Street et al., 2013; Wesołowska et al., 2006). Studies on various *Cichorium* species have identified polyamines, sterols, flavonoids, phenolic acids, anthocyanins, derivatives of hydroxycinnamic acid, sesquiterpene lactones, triterpenoids, norisoprenoids, and coumarins in the aerial parts of the plant (Hazra et al., 2002; Kisiel and Michalska, 2002; Krebsky et al., 1999; Mulinacci et al., 2001; Papetti et al., 2008; Tuzlaci and Doğan, 2010). Polysaccharides such as homogalacturonan, rhamnogalacturonan-I, cellulose, xyloglucan, heteroxylan, and glucomannan have also been found in the leaves. When discussing the phytochemicals found in chicory, it is essential to highlight the significant presence of inulin in its roots (El-Kholy et al., 2020). Also in the study by Piccolella et al., emphasizes the existence and diverse distribution of chlorogenic acids and flavonoids in chicory, underscoring the intricate nature of secondary metabolites in this particular plant species (Piccolella et al., 2024). The impact of inulin on wound healing is also a subject that warrants further investigation. Studies evaluating the biological activity of whole plant extracts of *C. intybus* have demonstrated hepatoprotective, antidiabetic, antioxidant, antibacterial, and anthelmintic properties (Gazzani et al., 2000; Papetti et al., 2002; Petrovic et al., 2004; Pushparaj et al., 2007).

Due to its widespread distribution, various parts of *C. intybus* have been used in traditional medicine around the world to treat a variety of diseases. The plant's aqueous root extract has been utilized to combat malaria in Afghanistan (Street et al., 2013), to treat warts in Iran (Bibi et al., 2017), to alleviate liver diseases and digestive problems in Poland, and to act as a mucilage and diuretic in Italy and Serbia (Mulinacci et al., 2001; Petrovic et al., 2004). Moreover, it has been reported to function as a pain reliever in Pakistan. The decoction of the plant's aerial shoots has been employed to counteract hemorrhoids and eczema, as well as for treating latex warts caused by cutting the tip of the petiole (Tuzlaci and Doğan, 2010; Yeşilada et al., 1999). In Turkey, a diverse range of ointments containing different substances have been developed and utilized for the aim of wound healing. Historically, this particular ointment was utilized to treat injuries on the necks of oxen that were crucial for agricultural tasks such as plowing fields. The roots and leaves of *C. intybus* are processed using traditional techniques and applied as a wound healing remedy among the local population. One of the related recipes includes the mixture of ash produced by burning the roots with butter, which is believed to be effective due to the particles/secondary products that may be released as a result of combustion. The second most well-known recipe involves mixing the dried leaves with *Anchusa leptophylla* roots, pine resin, and butter (Sezik et al., 1991).

Solvents of varying polarity are often employed to extract chemicals, yielding molecules that can be used in wound healing treatments. Depending on the nature of the solvent used, the resulting extract can contain compounds with different polarities and chemical structures (Süntar et al., 2012). In some studies, the methanolic extract of *C. intybus* was found to have a better wound healing effect, with beta-sitosterol potentially being the active compound responsible for this activity (Süntar et al., 2012).

In light of the substantial expansion of the pharmaceutical sector, it is worth noting that specific pharmaceuticals utilized in wound healing have the potential to give rise to difficulties throughout the course of treatment. The drugs in question are associated with low survivability and adverse side effects (Karukonda et al., 2000; Lodhi et al., 2016), there is a high demand for plant-derived medicines that are perceived to be safe, reliable, and effective (Komakech et al., 2019; Tyavambiza et al., 2022). Both natural and manufactured bioactive materials with wound healing potential possess antioxidant, chelation, and antibacterial capabilities, which may operate via one or more of these processes.

The study aimed to determine which prescription could be more effective for wound healing *in vitro*. By examining different parts of the plant and using various solvents, the study aimed to provide insights into the potential therapeutic benefits of the plant.

Materials and method

Collection and identification of plants

The specimen of *Cichorium intybus* (F15354; VPH) utilized in the research was gathered from Van, Turkey and taxonomically verified by Professor Fevzi ÖZGÖKÇE. The collection of samples commenced at the onset of the vegetative period. The identification technique employed the reference book titled "Flora of Turkey and the East Aegean Islands" and the VANF herbarium (Özhatay et al., 1999). The botanical components, including the flowers, roots, stem, and whole herba, were fragmented into small segments and subjected to a drying process under shaded conditions. The desiccated specimens were kept in a light-restricted environment until the investigations were conducted.

Preparation of extracts

The extraction investigations were conducted at the Phytotherapy Research Laboratory of Hamidiye Faculty of Pharmacy, University of Health Sciences Turkey. The various components of the plant, including the flower, stem, root, and other herbaceous portions, were individually pulverized. A total of 20 gs were obtained from the stem, 20 gs from the whole herba, 10 gs from the root, and 5 gs from the flower. Subsequently, each piece was individually supplemented with a quantity of solvent that was twenty times its weight. The solvents employed in this study included hexane, methanol (utilized for the initial extraction, followed by methanol usage on the subsequent day), and a 50 % (v/v) mixture of hexane and methanol. The samples were sealed and incubated overnight on a shaker at ambient temperature. The subsequent day, a rudimentary filtration procedure was conducted employing filter paper, and the solvent was subsequently evaporated using a rotary evaporator under reduced pressure (Heidolph/Heizbad Hei-VAP). The whole herba that remained was fragmented into smaller segments and subjected to incineration in order to obtain ash. The ash and extracts that were acquired were utilized *in vitro* experiments focused on wound

Table 2
The percentage of yields achieved through the extraction of plant parts.

Code	Part of Plant Used	Extraction Solvent	Extract Yield (%)
Sample 1	Stem	Hexane	1.364
Sample 2	Stem	Methanol	7.0285
Sample 3	Stem	Hexane - Methanol	0.848
Sample 4	Root	Hexane	0.76
Sample 5	Root	Methanol	6.978
Sample 6	Root	Hexane - Methanol	0.469
Sample 7	Flower	Hexane	2.32
Sample 8	Flower	Methanol	9.04
Sample 9	Flower	Hexane - Methanol	10.736
Sample 10	Whole Herba	Hexane	0.91
Sample 11	Whole Herba	Methanol	5.9305
Sample 12	Whole Herba	Hexane - Methanol	4.748
Sample 13	Whole Herba	–	–

Table 3
The amounts of polyphenolic compounds in the whole herba methanol extract calculated in mg/g extract.

Name of the phenolic compound	Concentration (mg/g)	Retention time (min.)
Chlorogenic acid	1964	12.432
Rutin	0.427	23.872
Quercetin	0.406	31.149

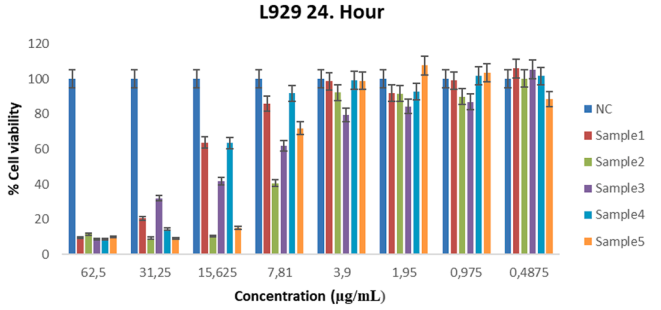


Fig. 1. Effects of extracts used in the study (NC: negative control, Sample 1–5) on L929 cells at 24 h.

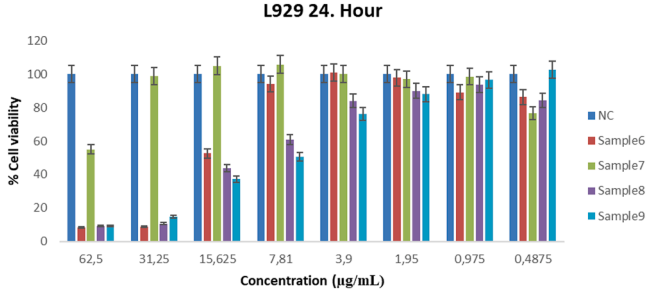


Fig. 2. Effects of extracts used in the study (NC: negative control, Sample 6–9) on L929 cell at 24 h.

healing.

Analysis of phenolic compounds by HPLC-PDA

Each extract used in the study's individual phenolic compound contents were screened for 15 standard phenolic compounds. We performed an analysis of these 15 standards, detecting them quantitatively in the extracts. Vanillic acid, caffeic acid, epicatechin, p-coumaric acid, salicylic acid, cinnamonic acid, rosmarinic acid, quercetin, chlorogenic acid, apigenin-7-O-glucoside, rutin, naringenin, 4-hydroxybenzoic acid,

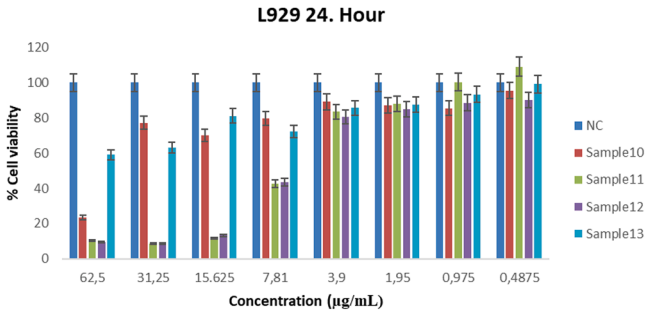


Fig. 3. Effects of extracts used in the study (NC: negative control, Sample 10–13) on L929 cell at 24 h.

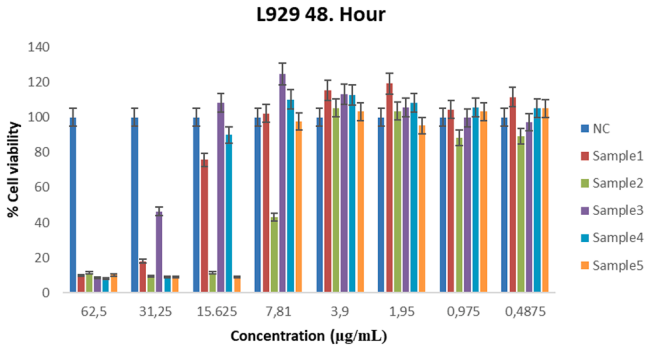


Fig. 4. Effects of extracts used in the study (NC: negative control, Sample 1–5) on L929 cell at 48 h.

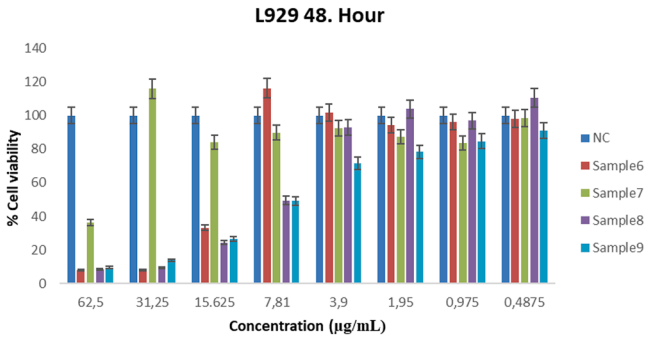


Fig. 5. Effects of extracts used in the study (NC: negative control, Sample 6–9) on L929 cell at 48 h.

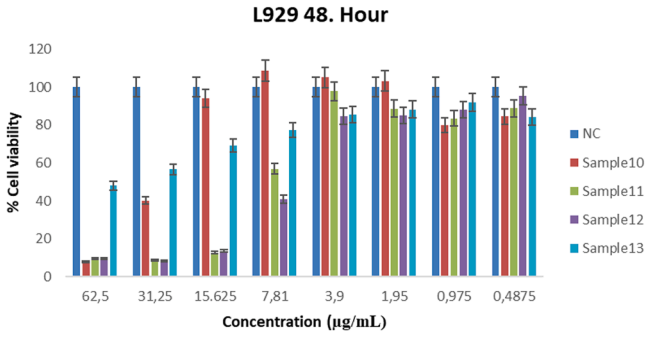


Fig. 6. Effects of extracts used in the study (NC: negative control, Sample 10–13) on L929 cell at 48 h.

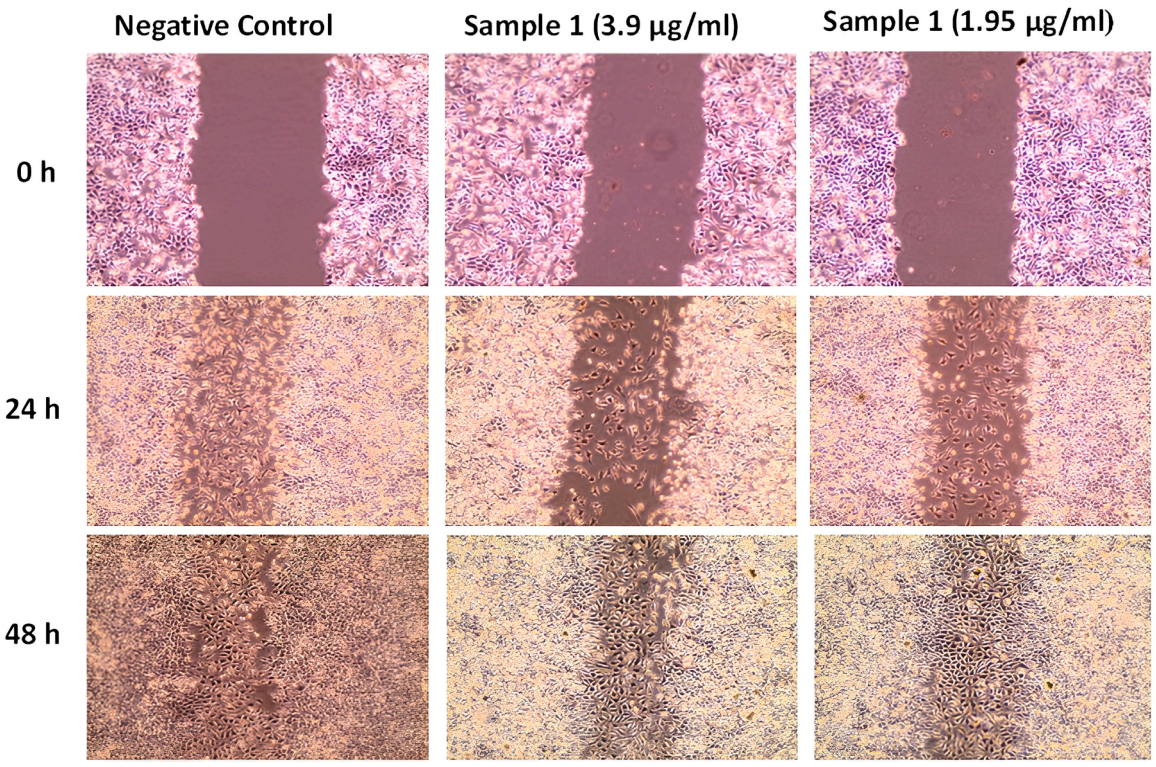


Fig. 7. Effect of Sample 1 extract on wound healing in L929 mouse fibroblast cells at different doses (3.9 and 1.95 µg/µL), 4x microscope image.

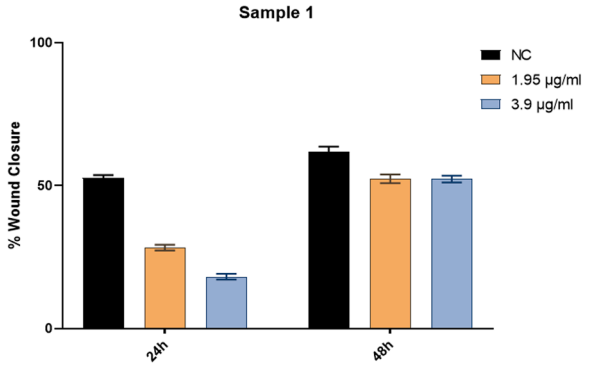


Fig. 8. Effects of Sample 1 extract on wound healing in L929 mouse fibroblast cells (NC: negative control).

gallic acid, and ferulic acid are used as standard compounds.

HPLC-PDA detector (Shimadzu Nexera-i LC-2040C 3D) was used to determine the amount of phenolic compounds present. The validation of the analytical method was carried out in accordance with the criteria proposed by the resolution of ANVISA RE number 899, 2003 (Anvisa, 2003). A phenylhexyl reverse phase column was used (3 µm, 4.6 × 150 mm; GL Sciences InterSustain). Table 1 displays the pump program (Ataseven et al., 2021). In the mobile phase, solvent A is 0.1 % formic acid in water, and solvent B is acetonitrile (Merck, HPLC grade). During the analysis, the mobile phase flow rate was set at 1 mL/min. The samples and standard injection volume are adjusted to 10 µL. The column temperature is set to 30 °C. For standards, stock solutions and extracts were prepared at a 1000 mg/L concentration. The results are calculated and expressed as mg/g extract.

In vitro cell culture studies

Taking advantage of *in vitro* scratch tests has proven to be an effective and economical method for assessing the migration and proliferation of cells *in vitro*, specifically during the process of wound healing. The utilization of two-dimensional *in vitro* cell migration experiments is employed to assess the capacity of cell populations to repopulate the region of wound (Jin et al., 2016). This study employed *in vitro* scratch tests and *in vitro* cell migration experiments to examine the wound healing effect of *C. intybus*.

The L929 mouse fibroblast cell line used in this investigation was procured from the cell stocks of the Animal Cell and Tissue Culture Laboratory at Tokat Gaziosmanpaşa University, Faculty of Pharmacy. All cell culture experiments were conducted within the confines of this laboratory.

The cryotubes containing cells were promptly thawed in a water bath set at 37 °C after being taken out from a liquid nitrogen tank maintained at a temperature of −196 °C. The contents of the tube were collected using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and antibiotics. The collected contents were then transferred to a centrifuge tube within a biosafety cabinet. Subsequently, the cells were subjected to centrifugation at a speed of 800 rpm for a duration of 5 min, while maintaining a temperature of +4 °C. Following the process of centrifugation, the liquid portion known as the supernatant was removed, and a new medium was introduced to the solid component, referred to as the pellet. Subsequently, the cell suspension underwent homogenization. The cells that had been homogenized were enumerated and subsequently placed to a growth dish of suitable dimensions. The culture dish was appropriately labeled and placed in an incubator set at a temperature of 37 °C, which was maintained by an oven with a controlled atmosphere of 5 % CO_2. The morphology of the cells was examined using reverse-phase light microscopy (Olympus, CKX41), and the culture media was refreshed every 48 h. Cells that achieved a confluency of 80–90 % were expanded by the process of passage.

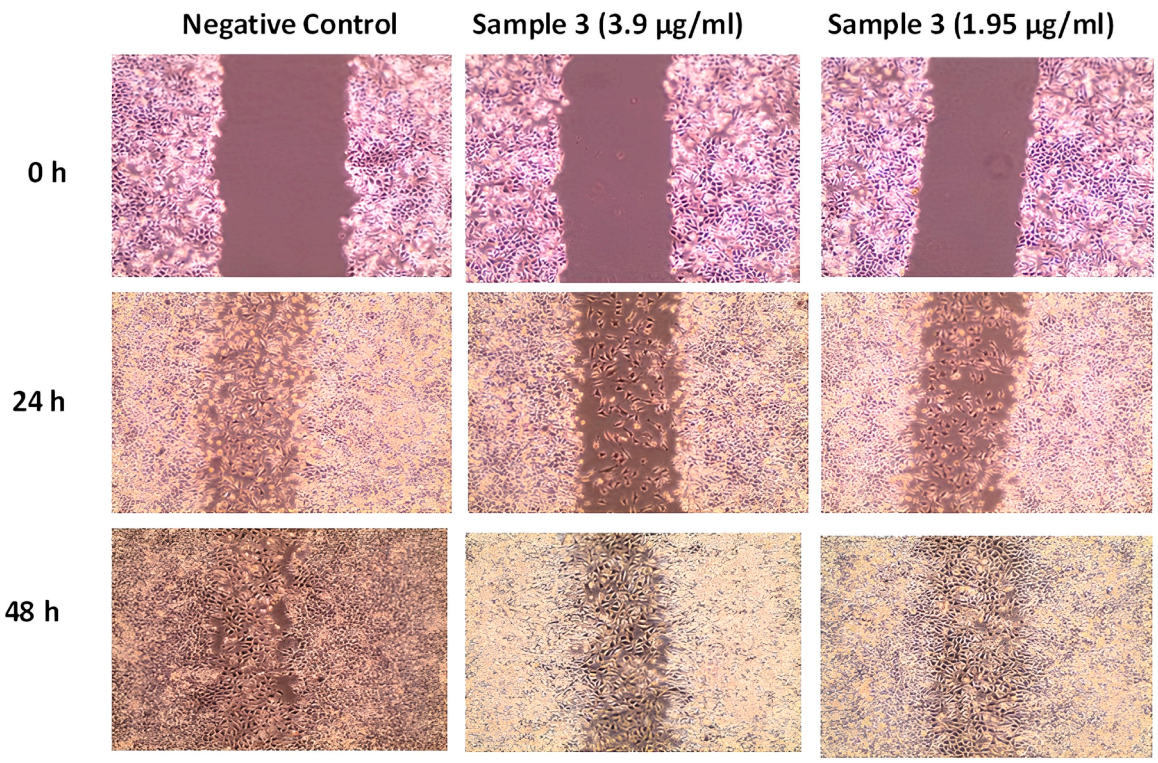


Fig. 9. Effect of Sample 3 extract used in the study on wound healing in L929 mouse fibroblast cells at different doses (3.9 and 1.95 µg/µL), 4x microscope view.

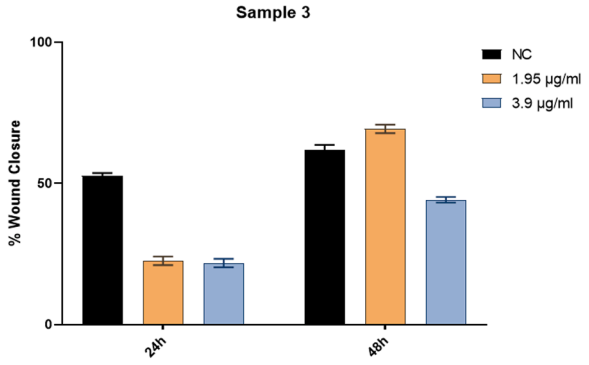


Fig. 10. Effects of Sample 3 extract on wound healing in L929 mouse fibroblast cells (NC: negative control).

Cell viability analysis

Following the removal of the utilized nutritional media from the cells, which covered approximately 80–90 % of the culture surface, the cells underwent a washing process using sterile phosphate buffer (PBS) devoid of calcium ions (Ca^{2+}) and magnesium ions (Mg^{2+}). This washing step was carried out at a temperature of 37 °C. Following that, the cells were treated with trypsin-EDTA solution at a temperature of 37 °C in order to facilitate detachment from the substrate. The cellular suspension was subjected to homogenization through the addition of a nutritional medium to the isolated cells, which was subsequently transferred to a centrifuge tube. During this phase, a volume of 200 µL of the cell suspension was transferred into a microcentrifuge tube for the purpose of determining the cell count. The cell suspension contained in the centrifuge tube was subjected to centrifugation at a speed of 800 rpm for a duration of 5 min at a temperature of +4 °C. Following the process of centrifugation, the liquid portion above the sedimented material, known as the supernatant, was carefully extracted. Subsequently, the solid mass of cells, referred to as the cell pellet, was subjected to homogenization within the appropriate medium. The process of cell counting was conducted utilizing a Neubauer Slide.

The laboratory supplies provided thawed cell lines, which were then plated in triplicate ($n = 3$) at a concentration of 5 × 104 cells/mL on 96-well culture plates. Subsequently, the cells were placed in a humid incubator with a 5 % concentration of carbon dioxide at a temperature of 37 °C for a duration of 24 h. Following the incubation period, plant samples of varying quantities were introduced to the cellular environment. The pertinent samples were dissolved in Dimethyl Sulfoxide (DMSO), and the maximum concentration of DMSO in the primary stock was determined to be 0.1 % in order to mitigate any potential harmful impact caused by DMSO. Sterile master stocks were generated and thereafter utilized to establish the initial working concentration for experimental purposes. A series of dilutions were performed in order to generate 6 distinct concentrations of the material, which were subsequently subjected to incubation with the cells for durations of 24 and 48 h. Following the completion of the incubation periods, an assessment of cell viability was conducted utilizing MTT (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide, a yellow tetrazolium salt). In the MTT test, the medium that was utilized was removed from the plate, and subsequently, the medium containing 10 % MTT (made in PBS at a concentration of 5 mg/mL) was introduced to the cells. The cells were then incubated in the dark for 3 h in a 5 % CO_2 incubator at 37 °C. After incubation, the medium containing MTT was withdrawn, and DMSO was added to dissolve the generated formazan crystals. The plate was subjected to analysis using an ELISA reader set to a wavelength of 570 nm, and the resulting absorbance readings were documented. The absorbance value of each sample exhibited a direct relationship with the absorbance value of the control cell group within its corresponding experimental set. Subsequently, the percentage of viability was calculated and visually represented on a graph.

Wound healing analysis

In order to evaluate the impact of *C. intybus* on the process of wound

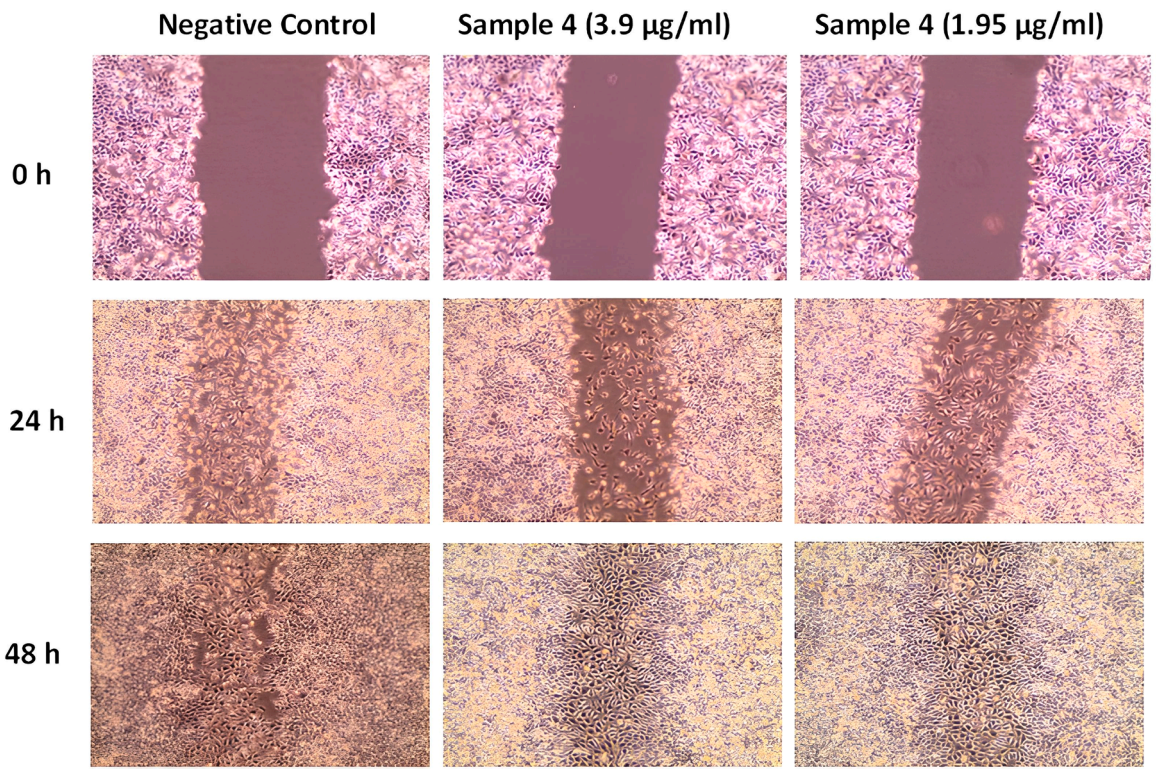


Fig. 11. Effect of Sample 4 extract used in the study at different doses (3.9 and 1.95 µg/µL) on wound healing in L929 mouse fibroblast cells, 4x microscope image.

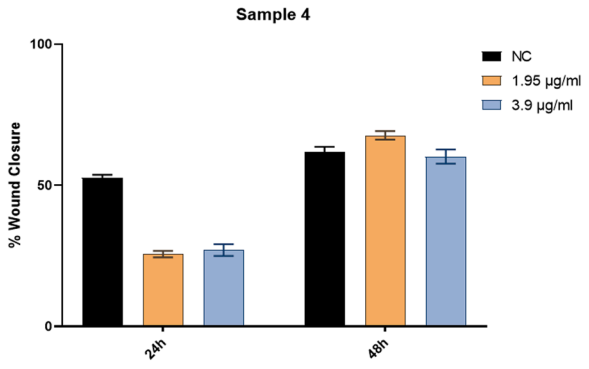


Fig. 12. Effects of Sample 4 extract on wound healing in L929 mouse fibroblast cells (NC: negative control).

healing, an *in vitro* scratch analysis was conducted utilizing the L929 murine fibroblast cell line. The process of wound healing is initiated by the body as a means to restore and mend damaged skin. This intricate process involves the activation of fibroblast cells in the dermis, which subsequently produce collagen in response to the inflammatory signals. This process facilitates the restoration of connective tissue, so enabling the regeneration of the epithelial cells in the outer layer of the skin. The phenomenon of wound healing encompasses a series of four distinct stages, namely hemostasis, inflammation, proliferation, and maturation. The examination of fibroblast response to materials plays a vital role in the process of wound healing. To assess the stimulating action of chemicals, researchers frequently employ the L929 cell line, which involves evaluating cell proliferation (Erden Tayhan et al., 2018).

The evaluation of the impact of the extracts on mouse fibroblast cells in the context of wound healing analysis was conducted through the utilization of the *in vitro* scratch assay. This particular method is widely recognized and renowned for its acceptance and cost-effectiveness. Mouse fibroblast cells were cultured in 6-well culture plates and permitted to attain a confluency level of 80–90 %. A "scratch" was generated on the cells after they reached the desired confluency using a sterile P200 pipette tip. Subsequently, the cells were exposed to the media containing the extract for an *in vitro* scratch assay. The cells were subjected to incubation with the corresponding extracts for a duration of 48 h. Subsequently, pictures were acquired at 24-hour intervals using an inverted microscope (Erden Tayhan et al., 2018; Liang et al., 2007; Lodhi et al., 2016; Oh et al., 2016). The images were subjected to analysis using ImageJ software in order to determine the rates of wound closure per unit area. The areas of the non-cellular wounds were identified and subsequently quantified by determining their total area. The areas obtained from ImageJ were imported into MS Excel software for further analysis. Subsequently, the proportion of the wound area relative to the entire area was computed as a percentage. The initial proportion of cells covered by the open spaces was also determined using MS Excel. The observed reduction in the surface area at various time intervals serves as an indication of the percentage of wound healing activity. The quantitative values obtained were compared to those of the negative control, and the rates of wound healing were determined. Wound healing experiments were conducted for all applications, encompassing both the utilization of extracts and their application in public settings, followed by further evaluations.

Statistical analysis

The statistical analysis of the data was performed using the SPSS package program, and One-Way Analysis of Variance (ANOVA) was applied. The results were presented as mean ± standard error. The level of statistical significance was set at $p < 0.05$ and $p < 0.01$.

Results

Extraction yields

Table 2 displays the extraction yields achieved from various sections

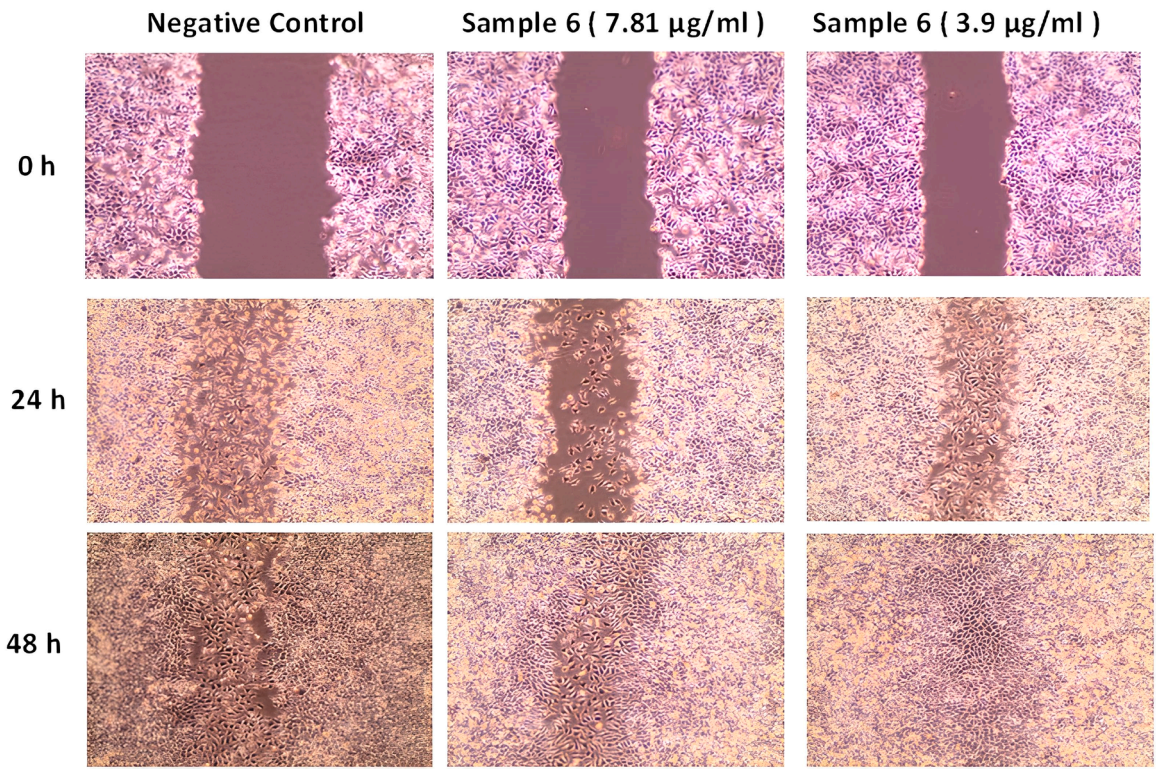


Fig. 13. Effect of Sample 6 extract at different doses (7.81 and 3.9 µg/µL) on wound healing in L929 mouse fibroblast cells, 4x microscope view.

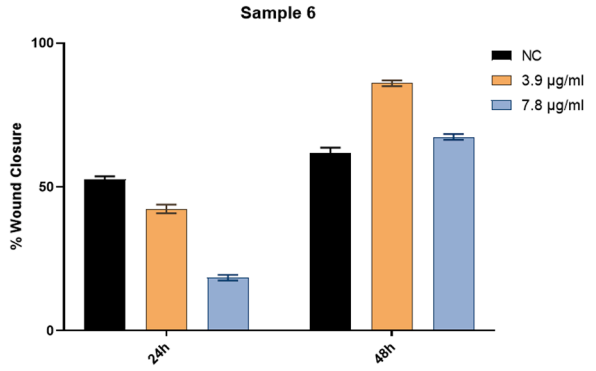


Fig. 14. Effects of Sample 6 extract on wound healing in L929 mouse fibroblast cells (NC: negative control).

of *C. intybus* using different solvents, presented as percentages. The code utilized for each extraction process and its matching plant component are also given. The findings indicated that the stem, root, and whole herba exhibited the most substantial extraction yields when subjected to methanol extraction. On the other hand, the extract with the highest yield for the flower component was derived from the methanol/hexane 50 % (v/v) mixture, as indicated in Table 2. Furthermore, Sample 13 was generated through the process of incineration, wherein the whole herba was subjected to combustion in order to yield the resulting ash.

Determination of phenolic compounds by HPLC-PDA

In HPLC investigation including 15 standard chemicals, we successfully identified three primary components in the *C. intybus* whole herba methanol extract. The substances mentioned are chlorogenic acid, rutin, and quercetin, respectively. According to Graphic 1 and Table 3, the major componend of the whole herba methanol extract is chlorogenic acid. The other chromatograms can be found in the supplementary materials .

Cell viability analysis

The L929 cell line was exposed to several extracts of *C. intybus* to assess their effects on the *in vitro* wound healing process. The determination of the optimal concentration for the scratch assay was initially conducted by evaluating cell viability using the MTT assay.

After conducting an examination of Fig. 1, it was noted that the methanol and hexane extracts (Samples 1–5) demonstrated a harmful impact within the first 24 h of interaction with L929 cells, particularly at the maximum dosage (15.625 µg/mL). Nevertheless, it was observed that the deleterious impact declined as the concentration of the extract dropped, resulting in enhanced cellular proliferation in comparison to the negative control. The results of this study exhibited statistical significance, as shown by a p-value of <0.05. In contrast, it was found that the extracts did not exhibit any cytotoxic effects at dosages equal to or below 3.9 µg/mL, as depicted in Fig. 1.

The results of the MTT examination performed on root and flower extracts (Samples 6–9) following a 24-hour incubation with L929 cells are illustrated in the graph displaying percent viability, as shown in Fig. 2. After careful analysis of the graph, it becomes apparent that Sample 7 had a significant increase in cell proliferation when exposed to a dose of 15.625 µg/mL. In addition, it was observed that both Sample 6 and Sample 7 exhibited a beneficial impact on cell proliferation when administered at lower concentrations (7.81, 3.9, and 1.95 µg/mL).

The assessment of cell viability was performed after a 24-hour period of exposure to L929 cells using extracts obtained from the entirety of the waste material (Samples 10–13), as depicted in Fig. 3. It is worth noting that Sample 11 demonstrated a substantial enhancement in cellular proliferation at lower dosages (0.975 and 0.4875 µg/mL). The obtained result demonstrated statistical significance at a significance level of $p \leq 0.05$.

Fig. 4 illustrates the cell proliferation outcomes acquired 48 h

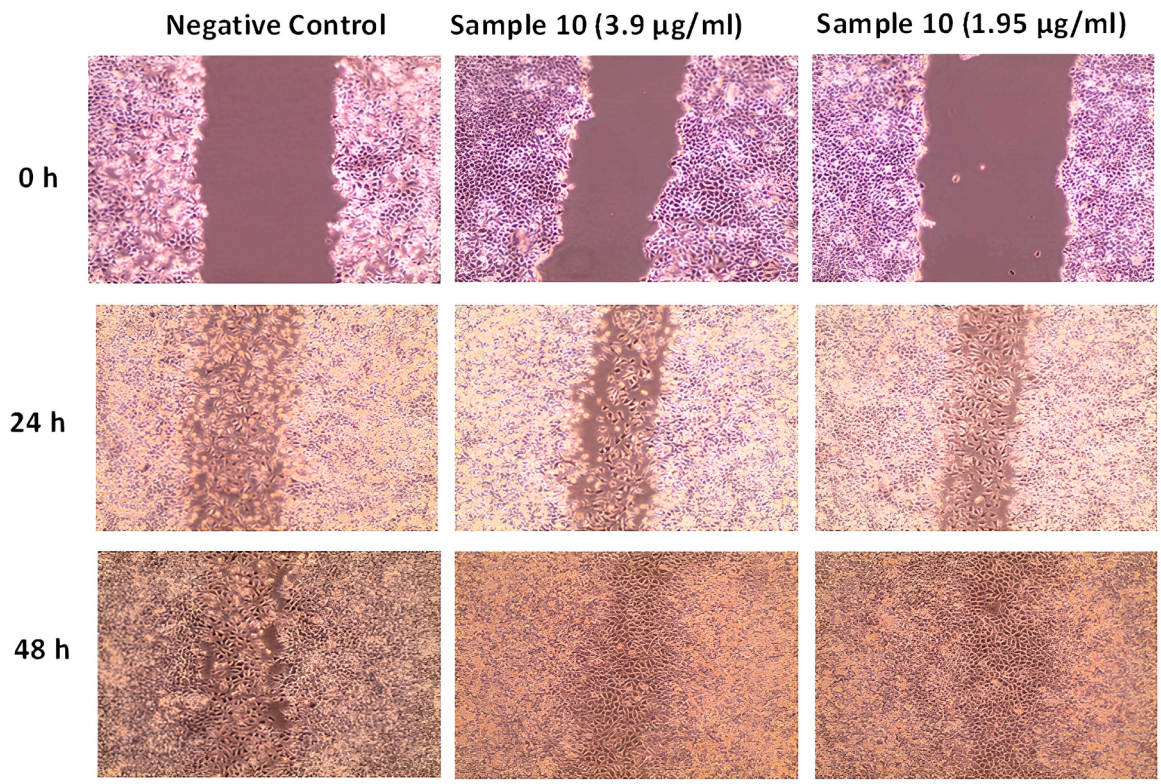


Fig. 15. Effect of Sample 10 extract used in the study on wound healing in L929 mouse fibroblast cells at different doses (3.9 and 1.95 µg/µL), 4x microscope image.

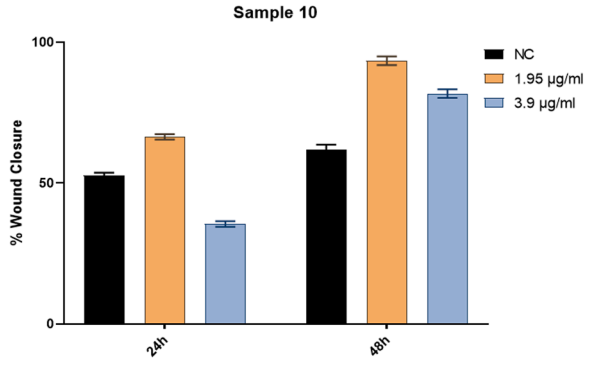


Fig. 16. Effects of Sample 10 extract on wound healing in L929 mouse fibroblast cells (NC: negative control).

subsequent to the interaction between L929 cells and the extracts (Samples 1–5). The results from Sample 3 indicate a noteworthy rise in cell proliferation when exposed to a quantity of 7.81 µg/mL. This observation is statistically significant, as indicated by a p-value of <0.05. Except for Sample 2, the other extracts seemed to have a favorable effect on cell growth when administered at modest concentrations. The study revealed that the extracts from Sample 1 and Sample 4 had beneficial impacts on cells when administered at doses of 3.9 and 1.95 µg/mL, respectively.

After conducting an analysis of Fig. 5, it was noted that the extracts (Samples 6–9) exhibited a rise in cell proliferation when exposed to a concentration of 7.81 µg/mL after 48 h of interaction with L929 cells. Moreover, it was determined that this outcome exhibited statistical significance at a level of $p < 0.05$. Nevertheless, the observed impact appeared to decline when administered at greater levels of 15.625 and 7.81 µg/mL. In general, it was observed that extracts derived from Samples 7–9 exhibited inhibitory effects on cell proliferation. Notably, Sample 8 shown a notable enhancement in cell proliferation at the lowest administered concentration (0.4874 µg/mL).

The MTT assay was performed subsequent to the incubation of extracts from Samples 10–13 with L929 murine fibroblast cells for a duration of 48 h. The outcomes of this analysis are visually shown in Fig. 6. Significantly, the hexane extract derived from the whole herba, specifically referred to as Sample 10, demonstrated a notable enhancement in cellular proliferation even at elevated doses, with the optimal dosage determined to be 7.81 µg/mL. In contrast, the extracts from Sample 11 and Sample 12 exhibited cytotoxicity at elevated quantities (15.625 and 7.81 µg/mL) with statistical significance ($p < 0.05$). Nevertheless, it was noted that the deleterious impact exhibited a decline when administered in smaller quantities.

The analysis of the viability graphs of the extracts (Samples 1–13) included in this study (Figs. 1–3) revealed that the concentrations of 3.9 and 1.95 µg/mL exhibited the highest efficacy in promoting wound healing. The observed doses had significant favorable effects on the survival and proliferation of cells, suggesting their potential effectiveness in facilitating the processes of wound healing.

Wound healing results

Through a comprehensive examination of cell viability tests conducted in this study, it was shown that many extracts, specifically Samples 1, 3, 4, 6, 8, 10, and 13, had favorable impacts on cell proliferation. The aforementioned extracts were regarded as potential agents that could promote the process of wound healing. As a result, the examination of wound healing was conducted by the utilization of the *in vitro* scratch test, employing the previously indicated extracts. The wound closure images acquired from the aforementioned analyses and the related computed percentages of wound closure are displayed in Figs. 7–18. In order to assess their wound healing capabilities, all studies were conducted using the two most efficacious doses identified using the MTT test. The results of these analyses would yield significant information regarding the wound healing capabilities of the discovered

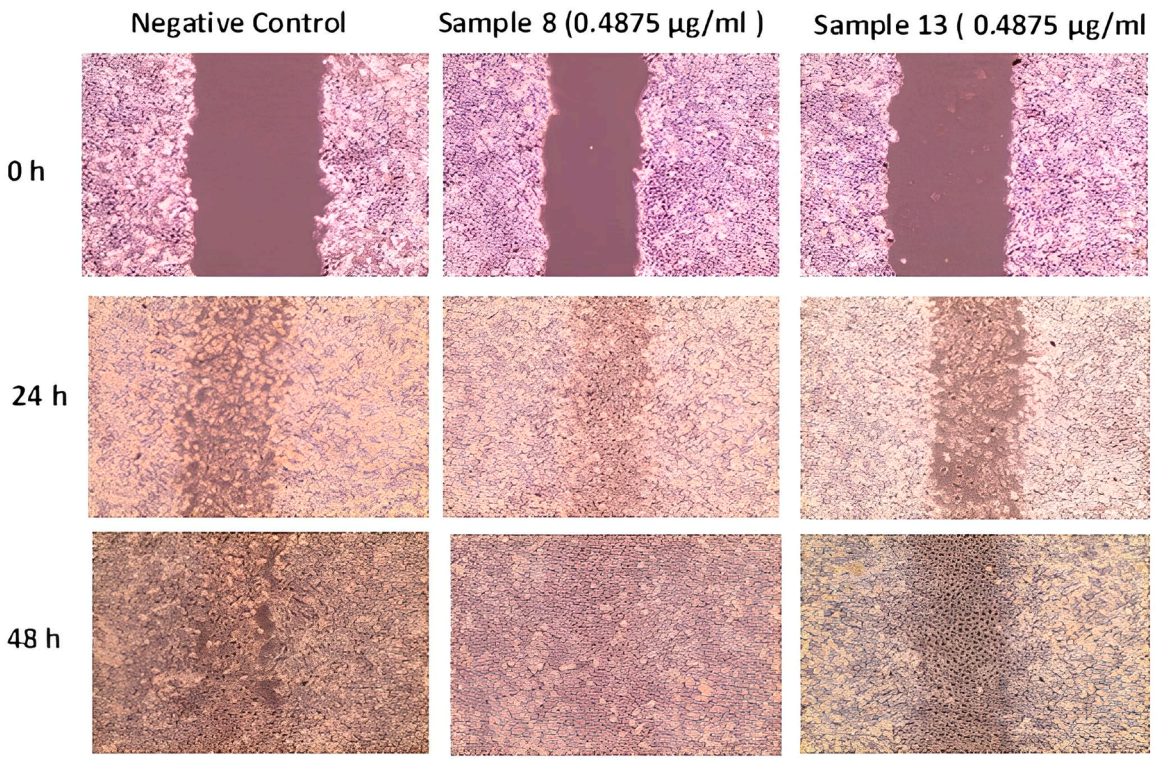


Fig. 17. Effect of Sample 8 and Sample 13 extracts used in the study on wound healing in L929 mouse fibroblast cells at a concentration of 0.4875 µg/µL, 4x microscope image.

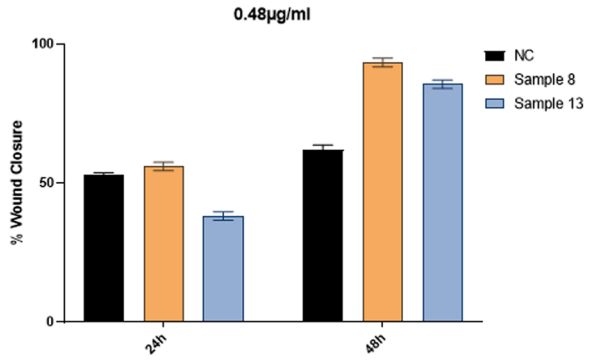
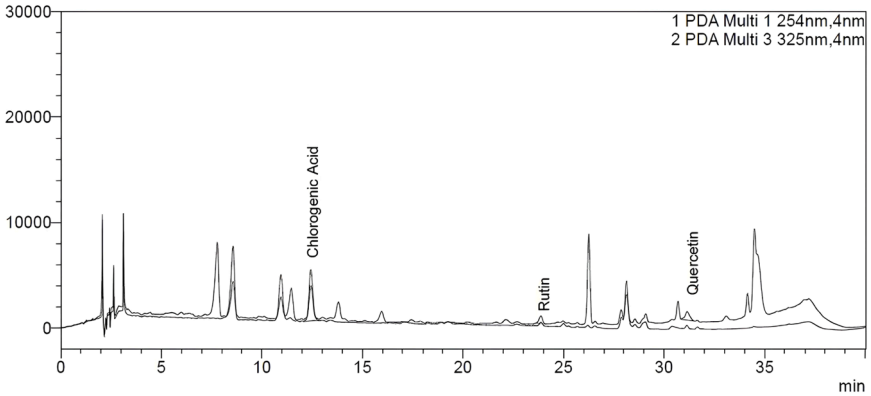


Fig. 18. Effects of Sample 8 and Sample 13 extracts on wound healing in L929 mouse fibroblast cells (NC: negative control).

extracts.

The evaluation of the effects of Sample 1 extract on the process of wound healing was conducted at two time points, specifically 24 and 48 h, following incubation. The concentrations used for the assessment were 3.9 and 1.95 µg/mL. The findings of this study revealed that the concentrations under investigation did not have any statistically significant impact on the process of wound healing, as evidenced by the comparison with the control group (Figs. 7 and 8). Despite showing positive effects on cell proliferation in previous analyses, the wound closure percentages did not exhibit any noticeable improvement with the application of Sample 1 extract at these specific doses.

The evaluation of the effects of Sample 3 extract on the process of wound healing was conducted by examining its impact at concentrations of 3.9 and 1.95 µg/mL following incubation periods of 24 and 48 h. Significantly, the proportions of lesions that exhibited healing at both concentrations following a 48-hour incubation period were seen to be greater compared to the control group. The observed difference was determined to have statistical significance ($p \leq 0.05$) based on Figs. 9



Graphic 1. Whole herba methanol exctract HPLC-DAD analysis chromatogram.

and 10. The results suggest that the administration of Sample 3 extract at the specified doses resulted in a significant improvement in wound healing when compared to the control group. The observed effects indicate that the extract from Sample 3 may have therapeutic benefits in facilitating the wound healing process. This finding justifies the need for additional research to investigate the underlying mechanisms of action.

The evaluation of the effects of Sample 4 extract on the process of wound healing was conducted by examining its impact at concentrations of 3.9 and 1.95 μg/mL following incubation periods of 24 and 48 h. The wound closure percentages at both concentrations were found to be higher than those of the control group after 48 h of incubation. Nevertheless, the statistical analysis conducted revealed that the observed difference was not deemed statistically significant, as demonstrated by a p-value greater than 0.05 (Figs. 11 and 12). While there appeared to be a certain degree of enhancement in wound closure when the Sample 4 extract was administered at a concentration of 1.95 μg/mL after a 48-hour period, the observed disparity did not attain statistical significance. Additional research may be warranted in order to comprehensively comprehend the potential impacts of the extract derived from Sample 4 on the process of wound healing. Furthermore, it may be beneficial to investigate alternative doses or durations of therapy to maximize the efficacy of the extract in promoting wound healing.

The evaluation of the effects of Sample 6 extract on the process of wound healing was conducted at concentrations of 7.81 and 3.9 μg/mL following incubation periods of 24 and 48 h. The wound closure percentages at both doses after 48 h of incubation were found to be significantly higher than the control group. Specifically, the difference in wound closure at the concentration of 3.9 μg/mL reached statistical significance ($p < 0.05$) (Figs. 13 and 14). According to the results, the application of Sample 6 extract, particularly at a concentration of 3.9 μg/mL after a duration of 48 h, resulted in a significantly accelerated wound healing process compared to the control group. The results emphasize the potential therapeutic efficacy of the extract derived from Sample 6 in facilitating the process of wound healing, hence warranting additional research to elucidate the underlying mechanisms through which it operates.

The study investigated the effects of Sample 10 extract on the process of wound healing. The extract was tested at doses of 3.9 and 1.95 μg/mL, and the incubation period lasted for 24 and 48 h. The percentages of wound closure at both concentrations during 48 h of incubation exhibited higher values compared to the control group. This disparity was found to be statistically significant ($p < 0.05$) as depicted in Figs. 15 and 16. Nevertheless, the incubation period of 24 h did not yield any statistically significant variation ($p > 0.05$). However, the group that had a 48-hour incubation with doses of 3.9 and 1.95 μg/mL exhibited a notable enhancement in wound healing when compared to the control group. The results of this study indicate that the extract derived from Sample 10 exhibits promising attributes for wound healing, particularly when administered at the specified dosages and incubation periods.

The evaluation of the effects of extracts from Sample 8 and Sample 13, at a concentration of 0.4875 μg/mL, on wound healing revealed that both extracts demonstrated a more pronounced impact on wound closure percentages in comparison to the control group after 48 h of incubation. A statistically significant difference was observed between the variables, as shown by the p-value of <0.05, as depicted in Figs. 17 and 18. The findings indicate that the extracts from Sample 8 and Sample 13, when administered at this particular dose and incubated for a duration of 48 h, exhibit promising potential in promoting wound healing.

The results depicted in Figs. 8, 10, 12, 14, 16, and 18 make the following conclusions:

The hexane extract obtained from the stem of *C. intybus* did not have any notable impact on the process of wound healing. Furthermore, it did not reveal any activity that could accelerate the healing process, as compared to the negative control.

The findings indicated that the application of Sample 3 extract, specifically the hexane/methanol extract derived from the stem of *C. intybus*, exhibited a beneficial effect on wound healing. Notably, this positive outcome was observed even when the extract was administered at a relatively low dosage of 1.95 μg/mL. The observed activity of the substance demonstrated an acceleration in the healing process when compared to the negative control.

The study revealed that the hexane extract of *C. intybus* root, specifically Sample 4 extract, exhibited enhanced wound healing properties compared to the negative control. Notably, even at a relatively low concentration of 1.95 μg/mL, the extract demonstrated accelerated wound healing effects.

The hexane/methanol extract of *C. intybus* root, as observed in Sample 6, exhibited limited efficacy in promoting wound healing at a concentration of 7.81 μg/mL. However, a notable enhancement in wound healing was observed at a lower dosage of 3.9 μg/mL. The observed activity of the substance exhibited an acceleration in the healing process when compared to the negative control.

The hexane extract of *C. intybus* herba, specifically Sample 10, shown diminished effects on wound healing when administered at a dosage of 3.9 μg/mL. However, the wound healing activity of this extract notably enhanced when the concentration was reduced to 1.95 μg/mL. The observed activity of the substance exhibited an acceleration in the healing process when compared to the negative control.

Both Sample 8, which is the methanol extract of *C. intybus* flower, and Sample 13, which is the ash of *C. intybus* whole herba, exhibited significantly enhanced wound healing properties when tested at a concentration of 0.4875 μg/mL. Both subjects exhibited an increase in the rate of healing compared to the negative control.

The results of this study indicate that extracts obtained from components of *C. intybus* had the ability to enhance the process of wound healing, particularly when administered at appropriate concentrations. Additional investigation into the fundamental mechanisms and potential clinical ramifications of these extracts may facilitate their application in the field of wound healing therapy.

The efficacy of the methanolic extract derived from the roots of *Cichorium intybus* in promoting wound healing has been demonstrated in the study conducted by Süntar et al. The researchers found that the dichloromethane (DCM) extract exhibited the highest efficacy in their investigation aimed at identifying the specific component responsible for the observed activity in the wound healing assays. The methanol extract was sequentially subjected to extraction using n-hexane, dichloromethane (DCM), ethyl acetate, and n-butanol. Based on the employed chromatographic techniques, it may be inferred that beta-sitosterol is the active compound responsible for the observed activity (Süntar et al., 2012). The findings of this inquiry exhibit a notable resemblance to the outcomes documented in the existing body of literature. The results of our experiment clearly indicate that hexane extracts derived from plant components or extracts obtained from a mixture of hexane and methanol exhibit the highest level of effectiveness. Consequently, it can be proven that the apolar molecules present in the extracts possess beneficial properties for the purpose of wound healing.

In a study conducted by Syed et al., the researchers administered a 100 mg/mL ethanolic extract to the subjects. The extract was topically given at concentrations of 50 mg/mL and 100 mg/mL in male Albino rats. It is important to note that just the extract, and not the ointment, was utilized in this experiment. The present work investigated the potential wound healing properties of the ethanolic extract derived from *C. intybus* in animal models. According to their findings (Syed et al., 2008), mice treated with the substance experienced complete wound epithelialization.

Discussion

Based on the results of the HPLC study, it can be concluded that the primary polyphenolic compound present is chlorogenic acid. Additional components include rutin and quercetin.

Within the scope of this research, chlorogenic acid is the main component in the extract and is therefore thought to be the main component in wound healing. In the study by Piccolella et al., plant components were examined and shown to be at similar rates to ours (Piccolella et al., 2024).

There are studies in the literature showing that chlorogenic acid has an important role in wound healing. Chlorogenic acid also affects wound healing in different forms, such as hydrogel, etc. Li et al. and Huang et al. conducted separate investigations that demonstrated the impact of chlorogenic acid on wound healing (Huang et al., 2023; Li et al., 2024).

The presented study adds innovation to the literature by demonstrating that, when used in appropriate doses, the chlorogenic acid contained in *C. intybus* extract has a positive effect on wound healing. In the study conducted by Süntar et al., total phenolic content was referenced. A comparative evaluation of traditional prescriptions from *C. intybus* for healing wounds was given, but the polyphenolic composition of *C. intybus* was not clearly given (Süntar et al., 2012). In Dalar and Konczak's study, polyphenolic compounds in the leaves of the plant were studied, but no information was given about parts such as the flower, stem, root, etc. (Dalar and Konczak, 2014).

In the present study, an investigation was conducted to compare the wound healing activities of the extracts designated as Sample 1, Sample 3, Sample 4, Sample 6, Sample 8, Sample 10, and Sample 13. The results revealed that, with the exception of Sample 1, all extracts demonstrated a wound healing accelerating effect at the indicated doses and higher. The results of this study provide evidence that the components of *C. intybus* possess the ability to promote wound healing.

The confluence of findings from several investigations, encompassing both existing literature and the present investigation, serves to bolster the substantiation of *C. intybus*' efficacy in promoting wound healing. Nevertheless, it is crucial to recognize that the determination of the active chemicals accountable for this particular activity necessitates more extensive and sophisticated investigations, as inferred from both prior research and the present study.

Conclusion

In summary, the cumulative body of evidence indicates that extracts derived from *C. intybus* has wound healing properties. It is novel to show that chlorogenic acid in C. intybus extract improves wound healing in adequate concentrations. The aforementioned findings emphasize the importance of *C. intybus* in the context of traditional medicine and pharmaceutical research, hence emphasizing the necessity for additional investigation into the active chemicals and mechanisms that contribute to its wound healing qualities. This research has the potential to contribute to the advancement of wound healing therapies by utilizing extracts or bioactive components derived from *C. intybus*.

CRediT authorship contribution statement

Berivan Kaçmaz: Resources, Investigation. **Duygu Misirli:** Resources, Investigation. **Seçil Erden Tayhan:** Investigation. **Zafer Ömer Özdemir:** Writing – review & editing, Conceptualization. **Mahfuz Elmastaş:** Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2024.100669.

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