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# Antioxidant and wound healing effects of comfrey (*Symphytum officinale*)

## Efectos antioxidantes y de cicatrización de heridas de la consuelda (*Symphytum officinale*)

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### ABSTRACT

This study explored the effects of comfrey (*Symphytum officinale*) on treating third–degree burns in a rat model, demonstrating promising results. A hot iron created standardized burns, after which three groups of rats received different treatments: no treatment (control), vaseline, or a 20% comfrey ointment. Histopathological assessments at the 7 days and 14 days marks showed accelerated healing in the comfrey–treated group compared to the control, indicating comfrey's potential for enhancing wound recovery. Additionally, comfrey's antioxidant capacity was evaluated using assays such as $Fe^{3+}$ and $Cu^{2+}$ reducing power and free radical scavenging activities (DPPH· and ABTS•⁺ assays). The IC50 values for DPPH· scavenging were 69.30 $\mu g \cdot mL^{-1}$ (water extract) and 46.20 $\mu g \cdot mL^{-1}$, while for $ABTS^{\bullet +}$ scavenging they were 77.00 $\mu g \cdot mL^{-1}$ and 69.30 $\mu g \cdot mL^{-1}$ (ethanol extract). These results confirm that comfrey exhibits significant antioxidant activity, likely contributing to its wound–healing properties. Overall, this study suggests comfrey as a safe traditional treatment option for burn healing, with its benefits likely stemming from its antioxidant activity. The results indicate this combination is a safe traditional medicine for clinical applications at proper doses.

**Key words:** Comfrey (*Symphytum officinale*); antioxidant activity; wound healing

### RESUMEN

Este estudio exploró los efectos de la consuelda (*Symphytum officinale*) en el tratamiento de quemaduras de tercer grado en un modelo de ratas, demostrando resultados prometedores. Se crearon quemaduras estandarizadas con un hierro caliente, después de lo cual tres grupos de ratas recibieron diferentes tratamientos: sin tratamiento (control), vaselina o una pomada con un 20% de consuelda. Las evaluaciones histopatológicas tanto a los 7 como a los 14 días mostraron una curación acelerada en el grupo tratado con consuelda en comparación con el grupo control, lo que indica el potencial de la consuelda para mejorar la recuperación de las heridas. Además, se evaluó la capacidad antioxidante de la consuelda utilizando ensayos como el poder reductor de $Fe^{3+}$ y $Cu^{2+}$ y las actividades de scavenging de radicales libres (ensayos DPPH· y $ABTS^{\bullet +}$). Los valores de IC50 para la eliminación de DPPH· fueron 69,30 $\mu g \cdot mL^{-1}$ (extracto acuoso) y 46,20 $\mu g \cdot mL^{-1}$, mientras que para la eliminación de $ABTS^{\bullet +}$ fueron 77,00 $\mu g \cdot mL^{-1}$ y 69,30 $\mu g \cdot mL^{-1}$ (extracto etanólico). Estos resultados confirman que la consuelda exhibe una actividad antioxidante significativa, lo que probablemente contribuye a sus propiedades de cicatrización de heridas. En general, este estudio sugiere la consuelda como una opción segura de tratamiento tradicional para la curación de quemaduras, con beneficios que probablemente provienen de su actividad antioxidante. Los resultados sugieren que esta combinación es una medicina tradicional segura para aplicaciones clínicas en dosis adecuadas.

**Palabras clave:** Consuelda (*Symphytum officinale*); actividad antioxidante; cicatrización de heridas

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## INTRODUCTION

Since ancient times, medicinal plants have been utilized to enhance human health and extend lifespan in various parts of the world. These plants are commonly used by millions, not only in areas with limited access to modern healthcare but also by urban populations in both developing and developed nations [1]. Türkiye is home to a diverse flora, with around 11,000 species. The local population utilizes this abundant plant life for various purposes [2]. It is well known that medicinal plants have antioxidant properties [3, 4] and may have greater potential for treating multiple biological disorders [5, 6, 7].

Comfrey (*Symphytum officinale*) is a medicine plant thought to be effective against wound site infection. The European Medicines Agency approves the use of comfrey on the skin. It was used as a reducing agent for the cytotoxic effects of antimicrobial active ingredients in the formula. It is also used for myogenic pain and arthralgia. Also, the therapeutic properties of comfrey, which are based on its anti–inflammatory and analgesic effects, stimulate granulation and support tissue regeneration. Thanks to those properties, it is used for wound treatment in folk medicine [8, 9]. Asimgil [9] reported that this medicinal plant was used to treat breast cracks and burn wounds.

Tissue damage caused by thermal, electrical, and radioactive agents is a burn. Burns range from some that can be treated and followed on an ambulatory basis to some that cause multi–organ failure. Burns cause some difficulties for individuals and societies because of mortality and morbidity [10]. Nearly 11 million people worldwide undergo medical treatment, and every year almost 300.000 people die because of burns [11]. Mortality rates due to burn wounds decrease in parallel with improvements in patient care and treatment [12]. Regardless of the etiology of burns, the tissue damage by burns is characterized by the denaturation of cell proteins, which becomes more severe depending on the cause and degree of injury [10]. A wound is divided into three degrees that depend on the depth of the wound; the manner of treatment is planned according to this degree: First–degree burns affect only the epidermis. Burns that affect the entire epidermis and superficial dermis are classified as second–degree burns. Third–degree burns, which extend through the epidermis and dermis, are full–thickness burns [13]. The aim of the treatment of burn wounds is quick re–epithelialization. It is required that the local agents used during the treatment of burn injury should be hygroscopic and easily applicable to speed up the quick re–epithelialization and prevent the possible wound infection [12], and dehydration of the wound [14].

Antimicrobial agents are routinely used to treat chronic and problematic wounds [14, 15, 16]. Comfrey was chosen for this study because of the above–mentioned characteristics. This study aims to reveal comfrey's antioxidant effects and evaluate its wound–healing effect according to ethnobotanical records.

## MATERIALS AND METHODS

### Plant materials

Comfrey was collected from Selçuk, İzmir province Türkiye (Latitude: 100 m) at the date of 08/24/2018. Whole plant parts were used for extractions. This plant and identified by Volkan Eroğlu, Ege University, Faculty of Sciences and Department of Biology, İzmir, Türkiye. The plant materials were authenticated by Herbarium Universitatis Aegaeensis Izmir (Herbarium No: 42708).

### Preparation of plant extract

A 100 g flowery comfrey plant, dried in the shade without direct exposure to sunlight and ground in a blender (Arzum AR-1111, Türkiye), was macerated in 80% ethanol (2×300 mL) for 2 days (d), with constant stirring at room temperature (between 21-27°C). The extracts were separated from the plant material by filtering twice through filter paper for increased efficiency. The filtered extracts were stored in glass flasks until further use. Following this, the ethanol extract was evaporated to dryness under reduced pressure using a rotary evaporator (Buch, R-210, Canada), resulting in a yield of 38.7 g. The dried extracts were then stored at -80°C (Arçelik, A++, Türkiye) until used in experiments. A 20% concentration of the dried extracts was applied to the burn wounds of rats (*Rattus norvegicus*) in the treatment group.

### Antioxidant activities

The total phenolic compounds in the Comfrey extracts were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton [17] using gallic acid as a standard phenolic compound. For determination of $Fe^{3+}$ reducing ability of comfrey, $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ reduction method was used. The spectrophotometer (Shimadzu UV-1800, Japan) was measured at 700 nm [18]. $Cu^{2+}$ reducing power was used as a second reducing ability method for comfrey. $Cu^{2+}$ reducing capability was performed according to the CUPRAC method [19] using the ethanolic neocuproine solution.

The absorbance of samples was recorded with a spectrophotometer (Shimadzu UV-1800, Japan) at 450 nm after 30 min [20]. DPPH radical scavenging activity of comfrey was performed according to the method of Blois [21] as described previously in detail [4]. The absorbance values of samples were recorded at 517 nm in a spectrophotometer (Shimadzu UV-1800, Japan) [22]. ABTS radical scavenging activity of comfrey was performed using the spectroscopic (Shimadzu UV-1800, Japan) method described by Huyut *et al.* [23]. The ABTS radical cation ($ABTS^{\bullet +}$) was acquired by reacting a 7.0 mM solution of ABTS with 2.45 mM $K_2S_2O_8$. The extent of decolorization is calculated as a % reduction of absorbance [24]. The percentage of metal chelating and radicals scavenging was computed using the following equation: SE (%) = [1×(As/Ac)]×100. Here, SE is radical scavenging effects, Ac is the absorbance value of control and As is the absorbance value of comfrey [25].

### Experimental animals

In this study, 42 female Wistar–Albino rats (180-200 g) (Mettler Toledo, XPR205, Switzerland) provided by the animal breeding laboratories of Çukurova University Experimental Application and Research Center are used. During the experiment, the animals were allowed food and water *ad libitum* (standard rat feed pellets supplied by the Forage Foundation). The animals were kept at constant room temperature (22°C) and they were fed. The Institutional Animal Ethical Committee of Çukurova University approved this study (Protocol no: 2017/6).

### Wounds

For general anesthesia, 10 mg·kg$^{-1}$ of Xylazine hydrochloride (Alfazyne®, 2%, Alfasan International, 3440 AB, Woerden, Holland) and 50 mg·kg$^{-1}$ of Ketamine (Ketalar®, Pfizer Pharma GMBH, Germany) were administered. Following anesthesia, the large areas on all rats' dorsal that the last rib joins with the thoracic vertebra and 1-2 lumbar vertebra's spinous process placed in there, and it comprises the vertebra posterior were shaved completely. The dorsal of rats was cleaned with povidone–iodine. A 50 g iron block of 15 mm diameter is used for the infliction of burns. The iron block was preheated for approximately 10 min until it reached a temperature of 70–100°C, and then it was applied without any additional pressure except the self–gravity of the block. The block was applied straightly for 10 seconds and a third–degree burn was created (full–thickness burns). The same procedure was repeated for each of the rats.

### Treatment protocol

Animals are randomly divided into three groups and treatment started 24 hours (h) after the burning procedure. Group 1 (control; n: 14): this group is not subjected to any kind of treatment. Group 2 (n: 14): vaseline only to observe the placebo effect of the vehicle; group 3 (n: 14) 20% (2 g extract + 8 g vaseline) comfrey ointment. A thin layer of ointment that covered the wound surface was applied once each d. Carprofen was given at 5 mg·kg$^{-1}$ to the wound area of all rats for analgesia once daily during the experiment.

### Measuring healing of burns

Diameters of burn wounds were recorded by measuring the length in mm with the help of a digital caliper (Mitutoyo 500-196-30, Japan) on the 1$^{st}$, 7$^{th}$, and 14$^{th}$ d after the burn injury. Measuring was carried out based on the borders of tissue that complete the re–epithelialization during the healing process. The diameter of the burn was recorded as 15 mm in all groups on the first d of measurement. During the 7$^{th}$ and 14$^{th}$ d, macroscopic findings related to healing burns were recorded. Any chemicals to remove accumulated drug residues on the burn wound area were not used. From the d the wound was formed, all the lesions were followed up continuously to the healing. Measurement of the wound area in the wound lines was photographed before applying the materials used on the wound during dressing changes in each group and recorded on the scale created by measuring the length.

### Histopathological evaluation

In this research, in all three groups, 7 rats were euthanized on the 7$^{th}$ d of the experiment, and on the 14$^{th}$ d of the experiment, the rest of the rats in the group were euthanized by high–dose anesthesia. Full–thickness tissue samples (15×15 mm), which included the whole burned sites, were harvested immediately after euthanasia each rat. After the collected tissue samples were fixed in 10% formaldehyde solution for the histopathological examination, paraffin blocks were prepared and samples that were 5 micron–thick were taken from them. These sections were then stained with Hematoxylin–Eosin (H&E) and histological findings related to ulcer, inflammation, epithelization, granulation tissue, and angiogenesis were evaluated under the light microscope (Olympus, BX53, Japan). Grading of histopathological parameters was done as in the literature [26].

### Statistical analysis

Statistical analysis was made through one–way analysis of variance (ANOVA) with multiple comparison tests (Tukey HSD). *P* value less than 0.05 was accepted as the level of statistical significance. The values were presented as mean ± standard error of the mean (SEM).

## RESULTS AND DISCUSSIONS

### Antioxidant results

In the present study, the antioxidant and antiradical potentials of comfrey were evaluated spectrophotometrically using a Shimadzu UV-1800 (Japan) and various bioanalytical methods, including $Fe^{3+}$–reducing antioxidant assay, cupric ion ($Cu^{2+}$)–reducing (CUPRAC) assay, DPPH• scavenging activity, and ABTS•+ scavenging activity. The results of comfrey's antioxidant activity are presented in TABLE I. The $Fe[(CN)_6]^{3-}$ to $Fe[(CN)_6]^{2-}$ reduction method was employed for the first time to assess the antioxidant capacity of comfrey [3]. As shown in TABLE I, comfrey extracts at a concentration of 10 µg·mL$^{-1}$ demonstrated significant $Fe[(CN)_6]^{3-}$ reducing activity, with these differences being statistically significant ($P$<0.01). An increase in the absorbance of $Fe[(CN)_6]^{2-}$ in the reaction mixture indicates enhanced reducing power, corresponding to higher complex formation. The reducing ability followed this order: Trolox (1.807 ± 0.002, $r^2$: 0.9938), α–tocopherol (0.579 ± 0.008, $r^2$: 0.9508), comfrey–ethanol (0.408 ± 0.028, $r^2$: 0.9494), and comfrey–water (0.357 ± 0.088, $r^2$: 0.9044). The CUPRAC assay, known for being cost–effective, stable, selective, and rapid, is suitable for assessing antioxidants regardless of their hydrophilicity or chemical structure [27]. The $Cu^{2+}$–reducing capacity of comfrey extracts and standard compounds is detailed in TABLE I. At the same concentration (10 µg·mL$^{-1}$), the $Cu^{2+}$–reducing ability ranked as follows: Trolox (0.618 ± 0.066, $r^2$: 0.9994), α–tocopherol (0.385 ± 0.004, $r^2$: 0.9206), comfrey–ethanol (0.135 ± 0.016, $r^2$: 0.9047), and comfrey–water (0.129 ± 0.005, $r^2$: 0.9718). A positive correlation was observed between the samples' $Fe^{3+}$– and $Cu^{2+}$–reducing capacities.

The radical scavenging capacities of comfrey extracts were assessed using DPPH• and ABTS•+ scavenging assays. The DPPH assay served as a preliminary test for evaluating the free radical scavenging activity of the extracts. Both comfrey extracts demonstrated effective DPPH• scavenging properties. As shown in TABLE I, the IC50 values for DPPH• scavenging activity followed this order: Trolox (IC50: 13.07 ± 0 µg·mL$^{-1}$) ≈ α–tocopherol (IC50: 13.32 ± 0 µg·mL$^{-1}$) > comfrey–ethanol (IC50: 46.20 µg·mL$^{-1}$) > comfrey–water (IC50: 69.30 µg·mL$^{-1}$). Similarly, significant ABTS•+ scavenging activity was observed, as reflected in TABLE I. The IC50 values for ABTS•+ scavenging were ranked as follows: comfrey–ethanol (IC50: 69.30 µg·mL$^{-1}$) > comfrey–water (IC50: 77.00 µg·mL$^{-1}$). For comparison, α–tocopherol (IC50: 6.66 µg·mL$^{-1}$) and Trolox (IC50: 6.86 µg·mL$^{-1}$) served as positive controls. A lower IC50 value indicates stronger scavenging activity, highlighting the notable electron–donating properties of the comfrey extracts for neutralizing free radicals. These findings demonstrate the potent DPPH• and ABTS•+ scavenging abilities of comfrey extracts.

***TABLE I***
**Determination of reducing power and radical scavenging of the same concentration (10 µg·mL$^{-1}$) of comfrey by ferric ions ($Fe^{3+}$) and cupric ions ($Cu^{2+}$) reducing capacities and the half maximal scavenging concentration ($IC_{50}$) DPPH• and ABTS•$^+$ scavenging methods**

| Antioxidants | $Fe^{3+}$–$Fe^{2+}$ reducing* | | $Cu^{2+}$–$Cu^{+}$ reducing* | | DPPH• scavenging** | | ABTS•$^+$ scavenging** | |
|---|---|---|---|---|---|---|---|---|
| | $\lambda_{700}$ | $R^2$ | $\lambda_{450}$ | $R^2$ | $IC_{50}$ | $R^2$ | $IC_{50}$ | $R^2$ |
| α–Tocopherol | 0.579 ± 0.008 | 0.9508 | 0.385 ± 0.004 | 0.9206 | 13.32 | 0.8769 | 6.66 | 0.8659 |
| Trolox | 1.087 ± 0.002 | 0.9938 | 0.618 ± 0.066 | 0.9994 | 13.07 | 0.9588 | 6.86 | 0.9997 |
| Comfrey–water | 0.357 ± 0.088 | 0.9044 | 0.129 ± 0.005 | 0.9718 | 69.30 | 0.9113 | 77.00 | 0.9605 |
| Comfrey–ethanol | 0.408 ± 0.028 | 0.9494 | 0.135 ± 0.016 | 0.9047 | 46.20 | 0.9349 | 69.30 | 0.9734 |

*: They were expressed as absorbance values, **: They were expressed as $IC_{50}$ values

## Macroscopic findings of the wound

After the operation of burning, the sizes of burning sites were measured in mm at different time intervals: 1st, 7th, and 14th d, respectively. On the first of measurement, the diameter of the burn was recorded as 15 mm in these groups. The differences between the changes related to healing of the burning sites between the 7th and 14th d of measurements were calculated and the healing rates are given in FIG 1. The one–way ANOVA was used to evaluate the healing rates of all the groups that were found by measuring the burning sites on the 1st, 7th, and 14th d and by calculating the difference between them statistically. Statistical significance was found among the groups. The maximum healing rates found were ordered as follows: group 3 > group 2 > control (FIG. 1). Additionally, the burning sites were photographed immediately after the operation of burning and on the 7th and 14th d of the experiment, and the changes in the diameter of burns were recorded (FIG. 1). During the healing process, wound infection was detected in the wound site in only one of the rats that belonged to the control group and it was excluded from the study. There were no other abnormal findings detected during the study.

## Histopathological findings

Parameters of histopathological lesion and healing of burn are presented in TABLE II. In the histopathological evaluation of the case study of the 7th d of the experiment that belongs to the control group, on the surface crust layer, and under this layer, inflammation was detected. On the 14th d, it was seen that on the surface crust layer, inflammation under this layer and epithelialization had started (FIGS. 2, A – D). In the histopathological evaluation of the case sections of the 7th d of the experiment that belongs to group 2, it was seen that epithelialization had started (FIGS. 3A and 3B). It can also be seen that epithelialization continues on the 14th d of the experiment (FIGS. 3C and 3D). In group 3, epithelialization was completed on the 7th d of the experiment (FIG. 4A). Complete epithelialization was observed (FIG. 4B). Complete epithelialization and granulation tissue under epithelialization can be seen (FIG. 4C). Through the 200× magnification, granulation tissue and proliferous vascular structures are seen (FIG. 4D).

**TABLE II**
**Histopathological parameters of burn healing on the 7th and 14th days of study**

| Histopathological parameters | Group 1 (control) | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|---|
| | Days | | Days | | Days | |
| | 7th | 14th | 7th | 14th | 7th | 14th |
| Ulcer | +++ | +++ | +++ | ++ | +++ | + |
| Inflammation | +++ | +++ | +++ | ++ | +++ | - |
| Epitelization | - | + | ++ | +++ | +++ | +++ |
| Granulation tissue | - | + | + | ++ | ++ | +++ |
| Angiogenesis | - | + | + | ++ | ++ | +++ |

Ulcer (presence +, absence - ), inflammation (absence - , mild +, moderate ++, intense +++), epitelization (absence - , mild +, moderate ++, intense +++), granulation tissue (absence - , mild +, moderate ++, intense +++), angiogenesis (absence - , mild +, moderate ++, intense +++)

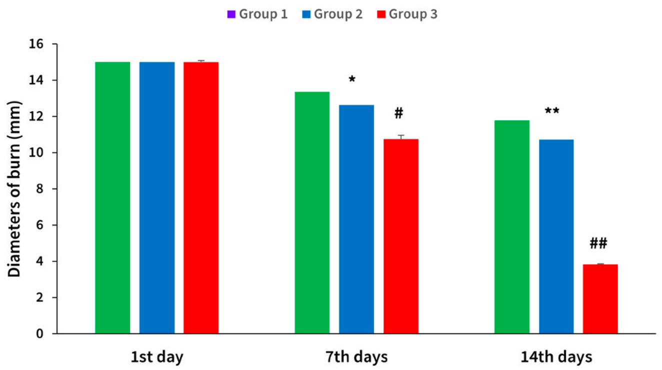


**FIGURE 1. *Statistical significance between group1, group 2, and group 3 (*P*<0.05), **: statistical significance between group 1 and group 2 (*P*<0.05), #: statistical significance between group1, group 2, and group 3 (*P*<0.05), ##: statistical significance between group 1 and group 2 (*P*<0.05)**

Phenolic compounds play a crucial role in medicinal plants due to their ability to scavenge free radicals, attributed to their hydroxyl groups [28]. At a concentration of one milligram each, the water and ethanol extracts of comfrey contained 20.0 and 27.6 µg of phenols measured as gallic acid equivalents, respectively. These compounds are believed to contribute directly to antioxidant activity [29, 30]. In this study, comfrey's antioxidant capacity was evaluated using assays such as $Fe^{3+}$ and $Cu^{2+}$ reducing power and free radical scavenging activities (DPPH· and ABTS•$^+$ assays). The $IC_{50}$ values for DPPH· scavenging were 69.30 µg·mL$^{-1}$ (water extract) and 46.20 µg·mL$^{-1}$, while for ABTS•$^+$ scavenging they were 77.00 µg·mL$^{-1}$ and 69.30 µg·mL$^{-1}$ (ethanol extract). These results show that comfrey exhibits significant antioxidant activity, likely contributing to its wound–healing properties. Overall, this

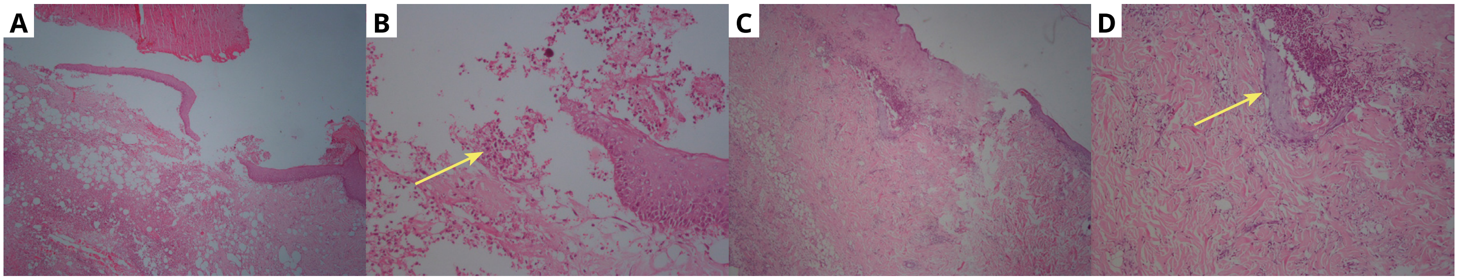


**FIGURE 2. Histopathological appearance of group 1' sections taken on the 7th and 14th days of the experiment, (A) Ulceration and cruts layer are partly detected on the 7th days of the experiment 40× H&E, (B) Active inflammation cells (arrow) are seen but regeneration is not seen 200× H&E, (C) Cruts on the surface and partial epithelialization are observed on the 14th days of the experiment 40× H&E, (D) Cruts on the surface and partial epithelialization (arrow) are seen 200× H&E**

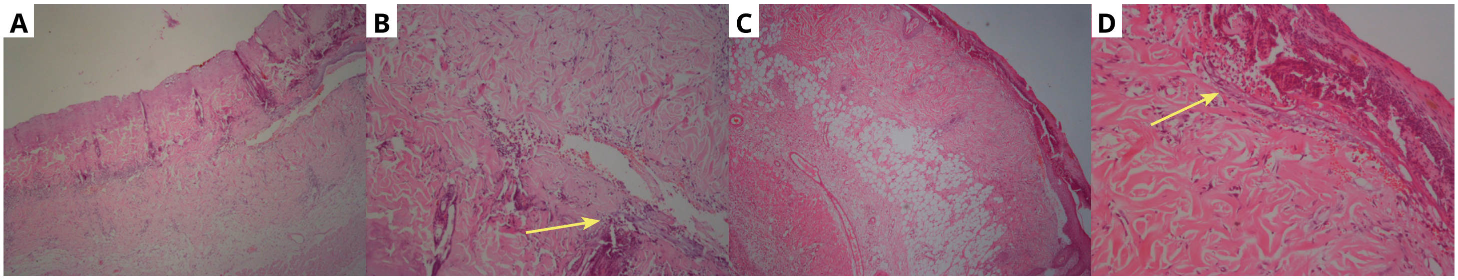


**FIGURE 3. Histopathological appearance of group 2's sections taken on the 7th days and 14th days of the experiment. (A) Cruts on the surface and under this surface partial epithelialization are observed on the 7th days of the experiment 40× H&E. (B) Cruts on the surface and under this surface partial epithelialization (arrow) are seen 200× H&E. (C) Cruts on the surface and partial epithelialization are observed on the 14th days of the experiment 40× H&E. (D) Epithelialization area (arrow). There was partial epithelialization 200× H&E**

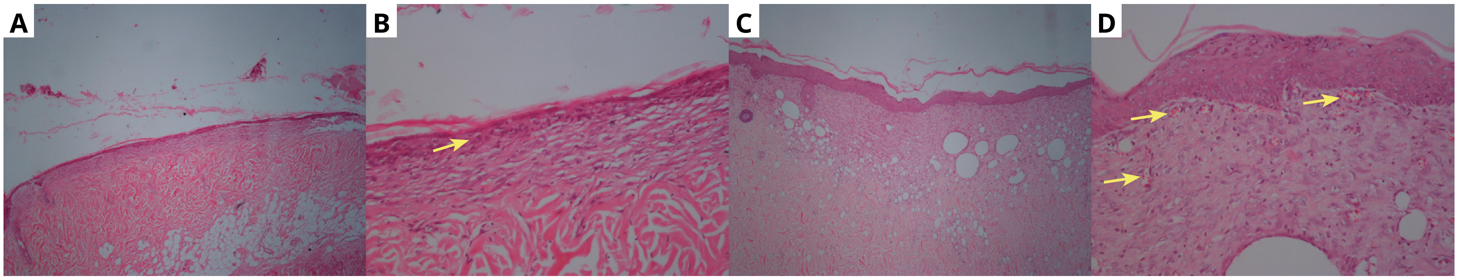


**FIGURE 4. Histopathological appearance of group 3 sections taken on the 7th days and 14th days of the experiment. (A) Complete epithelialization was observed on the 7th day of the experiment 40× H&E. (B) Complete epithelialization was seen 200× H&E. (C) Complete epithelialization and granulation tissue under epithelialization were seen 40× H&E. (D) Complete epithelialization, granulation tissue under epithelialization and proliferous vascular structures (arrow) can be observed 200× H&E**

study suggests comfrey as a safe traditional treatment option for burn healing, with its benefits likely stemming from its antioxidant activity.

Burn that causes morbidity and mortality is one of the severe traumas with which the body is faced. Burn is a severe health problem that causes minor injuries treated as ambulatory treatment, physiological changes in the tissue, hypovolemic, infection, alteration in body image, organ loss, and even death. When the skin, which forms a barrier to protect the body, is exposed to heat between 40–44°C, tissue integrity is damaged and the burning wound is shaped. Burns are graded according to the types of agents and the duration of exposure and the treatment is also planned according to these degrees. First–degree burns that affect only the epidermis require ambulatory care and therapy. It does not cause severe complications [31].

Second–degree and further degrees of burns require medical attention. The aim of the treatment of burn wounds is quick re–epithelialization. Burning skin which is the first barrier against infections makes the body unguarded against the attacks of microorganisms. It is a common complication that a burning wound gets infected and goes into sepsis in the burning cases [31, 32]. 75% of the causes of the death of the patients who burnt are infections [33]. It is recommended to use many antiseptic, antibiotic, and topical antimicrobial agents to protect the wound, which is open to infections [32, 34]. The most commonly used topical antimicrobial agent for this purpose is silver sulfadiazine (1%). It is known that among all medicines that can be used for this purpose, it is the one that has the least side effects. But when the re–epithelialization starts in the burning sites, it slows down the speed of the re–epithelialization. Because of this reason, it shouldn't be used at that time [34, 35, 36, 37]. It cannot be penetrated to eschar, which is a limiting factor in terms of its usage on burning wounds [11]. Using antibiotics during the treatment of a patient who is burnt is contradictive [34]. Even though their burning site is extended, prophylactic antibiotics during the treatment of each patient shouldn't be used routinely. By this means, the emergence of infections that are resistant to antibiotics can be prevented. But if the patients have both burnt and open wounds and infected and dirty wounds, they should use prophylactic antibiotics [38].

Infection of the burning site is the most important complication and necrotic tissue creates a suitable environment for the reproduction of the microorganisms. To control infection in cases of burning, it is required to strengthen debridement, which distracts infarct, strengthens immune response, ensures adequate nutrition, and uses topical, systemic antimicrobial agents. When most topical antimicrobials kill pathogens in the burning site, they can also kill fibroblasts and keratinocytes, which are reproduced during the healing period. It causes a delay in the healing of burning and it increases the risk of infection [36, 37, 38]. Several strong antimicrobial agents are not suitable for use in the treatment of burn injuries for a long period because of their cytotoxic effects [39].

In today's world, modern medicine uses herbs in the treatments, which have been used traditionally over the centuries [8, 39]. Phototherapeutic agents are used commonly for the healing of cutaneous wounds [40]. *Aloe vera* essence, which is one of the herbal medicines used commonly in the treatment of wounds, has a lot of bioactive components that enhance wound healing. Thanks to its certain features such as being a blood flow enhancer, moisturizer, antimicrobial, and anti–inflammatory, it heals wounds. It is reported that it can be an effective choice of treatment for first–degree and second–degree burns, shortening the time required for re–epithelialization and the time required for healing [41, 42, 43]. However, some studies are showing that certain substances taken from *Aloe vera* have negative effects on angiogenesis [44]. *Hypericum perforatum* is commonly used by society for wound treatment. It is reported that its wound–healing activity results from the increase in the fibroblastic activity and synthesis of collagen [45]. However, it is noted that it inhibits hypericin angiogenesis, which is one of the active substances of *Hypericum perforatum* [46].

Comfrey (*Symphytum officinale*) has been used by people since ancient times to alleviate pain and heal wounds. It is reported that allantoin, which is found in its structure, enhances the organism's immunity, has antioxidant and anti–inflammatory effects, and accelerates wound healing [8, 9]. Spin–Neto *et al.* [47] reported in their study on the effects of *Symphytum officinale* on hematopoietic strength and bone density in rats that it enhanced bone formation. In another study by Smith and Jacobson [48], the formulations of *Symphytum officinale* L. were superior to placebo in reducing pain and stiffness and improving physical functions in the treatment of osteoarthritis. In their study, Mulkijanyan *et al.* [49] recommend species of comfrey for treating second–degree burns. In the present study, we compared the effects of the control group, treatments with vaseline alone, and the combination of vaseline + *Symphytum officinale* on experimentally induced third–degree burns in rats. Based on macroscopic and histopathological findings, wound healing was faster in the treatment groups compared to the control group. Among the treatment groups, the vaseline + *Symphytum officinale* group showed faster wound healing than the Vaseline–only group. Our findings support those of previous researchers.

## CONCLUSIONS

In this research, we evaluated the effect of *Symphytum officinale* in the treatment of experimental third–degree burns in rats. Macroscopic and histopathological findings show that the wound heals more rapidly in the *Symphytum officinale* + vasaline group than in the the others groups. According to the results of our research using comfrey, which has antioxidant properties medicinal plants, and comfrey enhances the healing of experimental third–degree burns in rats.


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### Conflict of interest

The authors state that they have no conflicts of interest.


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