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Echium italicum Plant Extracts Have Wound Healing Potential in Human Dermal Fibroblast (HDF) Cell

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Abstract

Plants have an important place in the development of drugs used in the treatment of diseases, as in many areas. The flower and leaf parts of the *Echium italicum* plant used in the study have been used in many studies, but no information has been found in the literature about the use of the root part in increasing cell viability and wound healing treatment. In this study, the effects of *Echium italicum* , one of the Echium species naturally growing in our country, on cell viability and wound healing were investigated *in vitro*. Extracts prepared with different polarity solvents from the root and aerial parts of the plant were applied to the human dermal fibroblast (HDF) cell line to evaluate their effect on cell viability and wound healing potential. Cell viability was determined with the CVDK-8 method, and the wound healing activity of the plant was determined with the scratch assay. In summary, the effects of the *Echium italicum* root extract on wound healing were examined, and the medicinal properties of the plant suggested in traditional medicine were scientifically evaluated.

Keywords: *Echium italicum* , cell viability, wound healing, HDF cell

Introduction

Echium italicum is a plant species belonging to the Boraginaceae family, native to the Mediterranean region. It grows naturally in the Aegean and Mediterranean regions of Turkey but can sometimes be found in other Mediterranean countries and Southern Europe. This plant grown in temperate climates, especially in calcareous soils and sunny areas. The biological characteristics of *Echium italicum* include an annual or biennial life cycle. Its stem grows upright and usually reaches a height of 30 to 60 cm. Its leaves are arranged in rows, long and narrow, and have hairy edges. The flowers are collected in long panicles and are mostly blue, purple or pink. The flowering period usually coincides with the spring and summer months (1).

Research into the medicinal potential of the *Echium italicum* plant has focused particularly on the flower and leaf parts of the plant. These parts have been associated with antioxidant, anti-inflammatory and

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wound-healing properties. For example, extracts of the plant may be effective in combating free radicals due to their antioxidant capacity and may alleviate inflammatory conditions due to their anti-inflammatory properties. Additionally, many studies have been conducted on the positive effects of *Echium italicum* flower and leaf extracts on skin cells and their wound-healing potential (2).

Plant roots are a vital structure that anchors the plant to the soil and helps it absorb nutrients from the soil. Roots also enable the plant to take in and store water, helping the plant survive dry periods. Roots can also produce hormones that regulate plant growth and development (3). From a medicinal point of view, many plant roots have long been used in traditional medicine. Roots generally contain the richest bioactive components of the plant and due to these properties, they have various positive effects on health. Root extracts have biological effects such as antioxidant, anti-inflammatory, antibacterial and antiviral. These can contribute to the development of drugs used in the treatment of various diseases (4).

Plant extracts are concentrated extracts obtained from various parts of plants (e.g. leaves, flowers, roots) and contain many bioactive compounds. These bioactive compounds are generally known as phytochemicals and play a role in the defense mechanisms of plants, growth regulation, and protection against environmental stresses. Plant extracts have long been used in traditional medicine and are considered an important source in the drug development process in modern medicine (5). The cellular effects of plant extracts vary depending on the bioactive compounds they contain. Various bioactive compounds, such as antioxidants, anti-inflammatories, anticancer agents, and antimicrobial compounds, exert various effects at the cellular level. For example, antioxidants protect cells from the harmful effects of free radicals by reducing cellular oxidative stress. Anti-inflammatories, on the other hand, alleviate inflammatory conditions by reducing inflammation. Anticancer agents can prevent the growth and spread of cancer cells, while antimicrobial compounds inhibit the growth of bacteria, viruses, and fungi (6).

The cellular effects and therapeutic potential of plant extracts have been the focus of many studies investigating their use in the pharmaceutical industry. Such studies help us understand how plant extracts act at the cellular level and identify potential therapeutic agents (7).

Human dermal fibroblast (HDF) cells are the most common cell type found in the skin and have a variety of functions. Fibroblasts play important roles in maintaining the structural integrity of skin tissue, promoting wound healing, and contributing to tissue regeneration. During the wound healing process, fibroblasts synthesize collagen and other extracellular matrix components that promote wound healing (8).

Wound healing is generally described as a three-phase process: inflammation, regeneration (proliferation), and remodeling. First, inflammation occurs at the wound site and clears damaged tissue and microorganisms. Second, during the regeneration phase, fibroblasts synthesize extracellular matrix components such as collagen and elastin in the wound site and stimulate tissue regeneration by forming new vessels. Finally, during the remodeling phase, the structural integrity of the tissue is restored, and excess matrix material is cleared (9).

The role of fibroblasts in wound healing is mediated by cell migration, proliferation, and production of extracellular matrix. These processes allow cells to come together to close the wound and repair damaged tissue. The effectiveness of fibroblasts in wound healing depends on several factors, including growth factors, cytokines, and extracellular matrix components (10).

Studies on HDF cells can contribute to our understanding of the molecular and cellular mechanisms of wound healing and the development of

new therapeutic methods targeting these processes. Therefore, studies on HDF cells and wound healing play an important role in clinical applications such as the treatment of skin diseases and wound healing (11). This study adopts a scientific approach to understand the potential health effects of *Echium italicum* root parts. The focus of the study was to evaluate the effects of *Echium italicum* root extracts on cell viability and wound healing *in vitro*.

Material and Method

Plant Collection and Storage Conditions: *Echium italicum* plant was freshly collected from Mustafakemalpaşa district of Bursa province and brought to the laboratory via cargo. The drying process of the roots was carried out at low temperature and in a dark environment to preserve the biological activity of the plant. The solvents used in obtaining the plant extracts were stored in storage cabinets at room temperature (25°C).

Preparation of Extracts: Three different extracts were obtained from the dried roots using polarity solvents such as acetone, methanol and H_2O_2. For the extraction process, 20 grams of plant material was weighed using a precision balance and 100 ml of acetone, methanol and H2O2 were added separately, mixed until a homogeneous mixture was obtained and filtered. The obtained extracts were concentrated using an evaporator at 55°C, 65°C, 95C for acetone, methanol and H2O2 respectively. The extracts were air dried and calculated in mg/g. They were stored at -20 °C for further studies (12).

Concentration Calculation: 20 mg of the extracts were taken from the sand and dissolved in 200 ul DMSO (Dimethyl sulfoxide) and subjected to sonification. After the concentrations determined from these extracts prepared as stocks (0,10 mg/mL, 0,25 mg/mL, 0,50 mg/mL, 1 mg/mL) were prepared, a homogeneous structure was obtained by passing through a 0.45 mm filter.

Cell Culture: Human dermal fibroblast (HDF) cells were propagated in DMEM medium (10% FBS, 1X L-Glutamine and 1X Penicillin/Streptomycin) (EcoTech Biotechnology, Türkiye) and then seeded into T25 flasks with 5 ml medium and incubated in a 5% CO2 oven at 37˚C. Cells were dissociated using trypsin every 2-3 days and cultured for 4 weeks. All cell culture studies were performed in a sterile laminar air flow cabinet. Appropriate conditions were provided for cell proliferation and passaging was performed when the cell density reached 60-80% confluency.

Cell Viability Assay: HDF cells were counted under a microscope and distributed equally in a 96-well plate so that $1.5x10^3$ cells would be seeded in each well, and then the plates were kept in the incubator for 24 hours. At the end of the incubation, plant extracts at different concentrations (0,10 mg/mL, 0,25 mg/mL, 0,50 mg/mL, 1 mg/mL) and negative (DMEM) and positive (10% DMSO) controls were applied to the cells prepared for the experiment and kept in the incubator for 24 hours. Three replicates were run for each experimental group to ensure repeatability. Cell viability was determined using the CVDK-8 kit (EcoTech Biotechnology, Türkiye). 50 µl (medium and 10% kit) of the CVDK-8 kit solution was applied to each well of the 96-well plate where the concentration application was made. Cells were incubated in the dark at 37°C. Measurements were taken every half hour on the Biotech Epoch device (13).

Wound Healing Test (Scratch Assay): To evaluate the effect of the plant extract on wound healing, HDF cells were counted and 75x104 cells were seeded into each well of a 6-well plate and kept in an incubator for 24 hours to ensure monolayer formation. Scratches were created on the cell surface attached to the ground using a 200 ul sterile pipette tip in a regular and standard manner. After 24 hours, wound closure

formations were observed and recorded with a Leica inverted microscope (14).

Statistical Analysis: The obtained results were visualized using GraphPad Prism 10 and ImageJ programs. Statistical analysis for all work packages was performed using two-sided Student's t-test with log transformed data, and p <0.05 values were considered statistically significant.

Results

Cell Viability Result: After incubating the acetone extract applied at various concentrations for 24 hours, the 0,10 mg/ml concentration of *Echium italicum* had no significant effect on cell viability, while other concentrations increased cell viability (Figure 1, p<0.05).

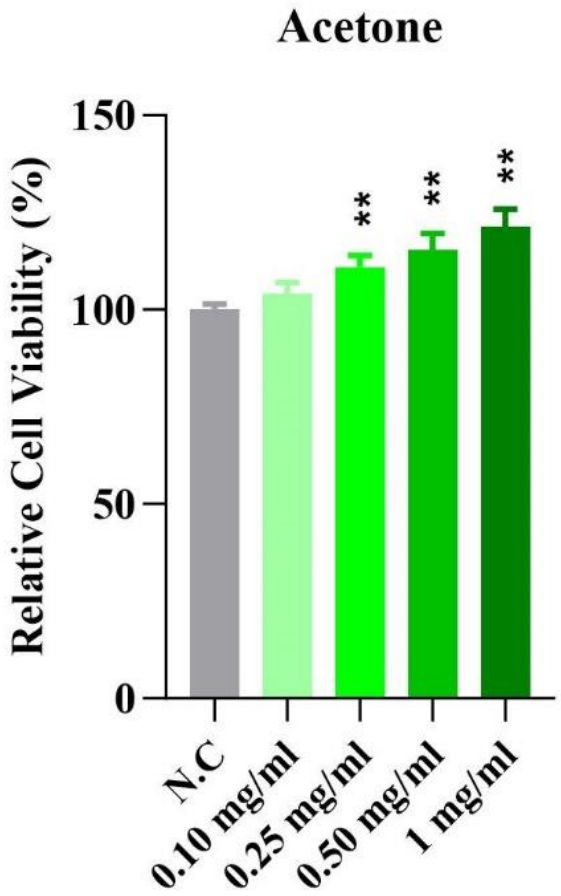


Figure 1. Effects of various concentrations of acetone extraction on HDF cells.

In the examination of the effects of the methanol extract of *Echium italicum* applied to HDF cells for 24 hours on cell viability, it was determined that the dose of 0,10 mg/ml did not have a significant effect, while other doses significantly increased cell viability (Figure 2, p<0.05).

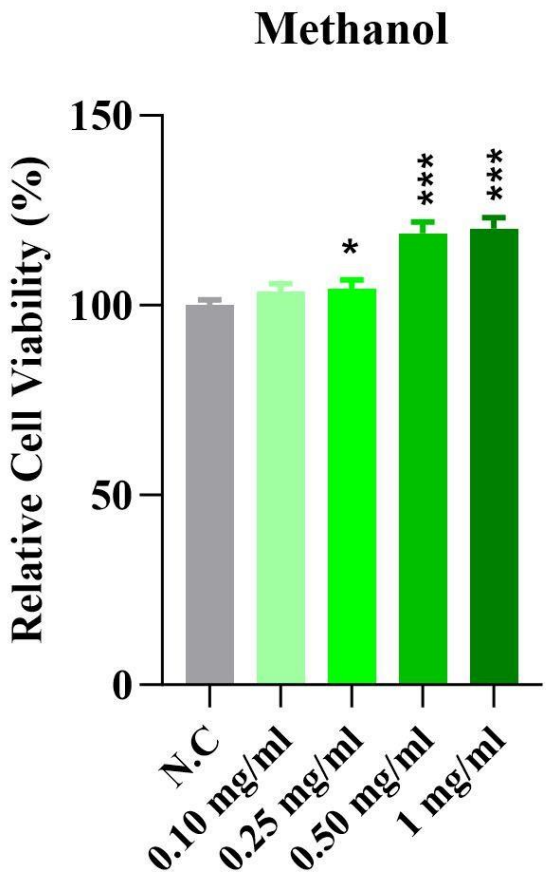


Figure 2. Effects of various concentrations of methanol extraction on HDF cells.

In the examination of the effects of 24-hour application of *Echium italicum* H_2O extract on cell viability of HDF cells, all applied concentrations of *Echium italicum* significantly increased cell viability (Figure 3, p<0.05).

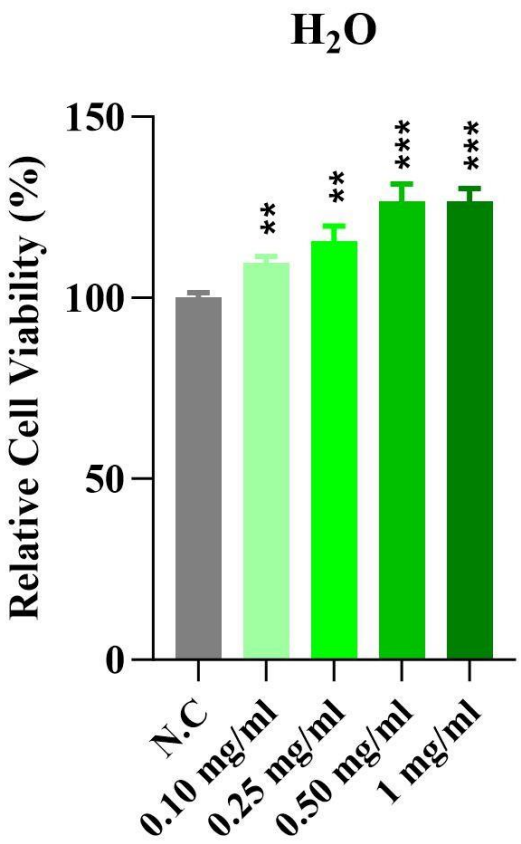


Figure 3. Effects of various concentrations of H2O extraction on HDF cells.

Wound Healing (Scratch Assay) Result: The effects of the obtained plant extract on wound healing were evaluated with the wound healing test. The lowest concentration of acetone extract (0,10 mg/ml) was selected and given to the cells. At the end of 24 hours of incubation, the wound closure status was observed under a microscope and photographed.

In the analysis of cell wound area closure (scratch test), while no significant increase in wound area closure was observed in the control group, it was determined that the wound area closed significantly in the group to which *Echium italicum* acetone extract was applied (Figures4,5,6).

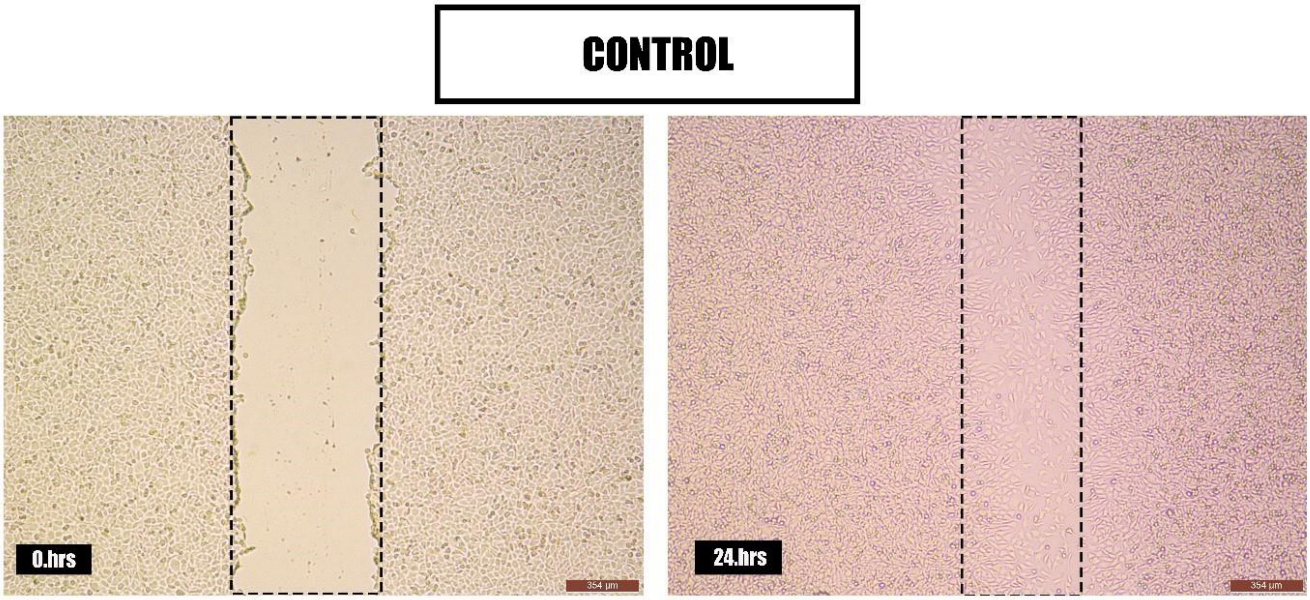


Figure 4. Control group 0-24th hour surface coverage image

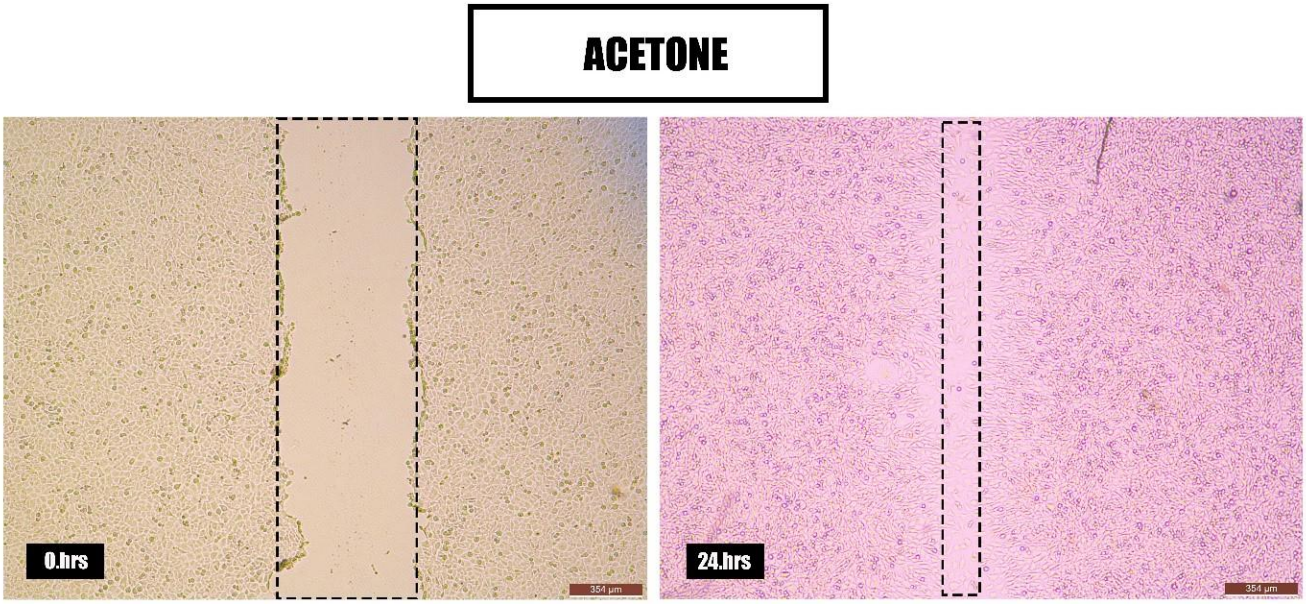


Figure 5. Acetone extraction group 0-24th hour surface coverage image

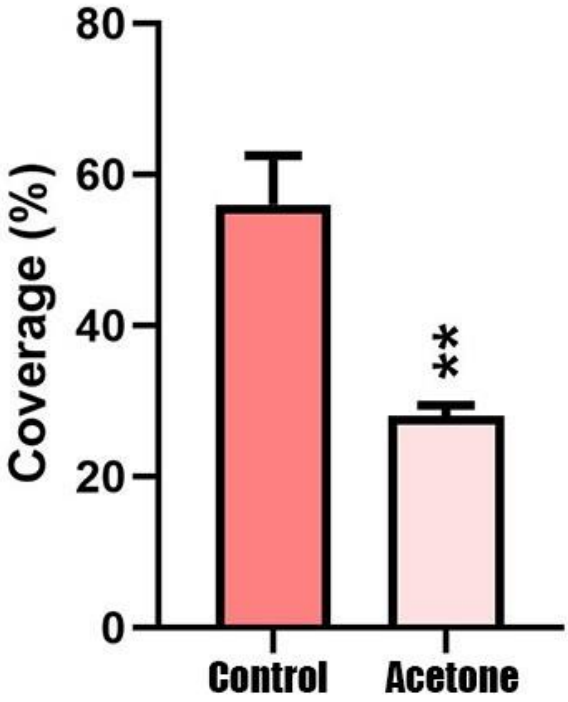


Figure 6. Comparison of wound opening area in acetone extraction application compared to control in HDF cell line.

Discussion

Echium italicum is a plant belonging to the Boraginaceae family and is widely found in the Mediterranean region. The root parts of this plant have been used for various purposes in traditional medicine (15). In this study, the medicinal properties of the *Echium italicum* plant recommended by traditional medicine were evaluated and the traditional use of Echium Sp. was brought to a rational basis. In addition,

the potential effects of the root extract of the *Echium italicum* plant on wound healing were examined. In the literature, the red roots of the *Echium italicum* plant are used especially in the treatment of burns (16). Based on these data, the effects of the plant on cell viability and wound healing using solvents not used in the literature were examined by following scientific methodology. The study and the results obtained revealed important findings that *Echium italicum* root extract can positively affect the wound healing process. First, the effect of the plant extract on cellular proliferation was evaluated and it was observed that it increased the proliferation ability of HDF cells when applied at different concentrations (Figure 1, 2, 3). This suggests that the plant extract can promote the regeneration of fibroblast cells. In addition, its effects on cellular migration, which plays an important role in the wound healing process, were examined. Scratch assay experiments showed that *Echium italicum* root extract increased the migration potential of HDF cells (Figure 4, 5, 6). In conclusion, this study reveals the positive effects of *Echium italicum* plant root extract on wound healing.

Studies show that phytochemical components such as naphthoquinones and flavonoids support the wound healing process mainly due to their antimicrobial properties that promote cell proliferation and granulation tissue and are responsible for wound contraction (17). It is considered that the high naphthoquinone content in *Echium italicum* roots may be responsible for the wound healing activity, probably by promoting cell proliferation and migration, and can form thick granulation tissue and re-epithelialization of wounds, but these results need to be supported by further studies.

Conclusions

The main purposes of analyzing crude plant extracts are to isolate bioactive agents either for direct use as drugs or to identify bioactive compounds that can be used as precursors for the preparation of semi-synthetic drugs. Numerous new wound healing drugs have been discovered from natural products in the past and new ones are being developed continuously. These natural products can play an important role in wound treatment by working together with traditional drugs, thus increasing their efficacy or reducing their toxicity (18,19). The results of our study indicate that *Echium italicum* plant extract has promising wound healing activities *in vitro*. It is expected that more comprehensive studies will be conducted in the future to obtain therapeutic implications of these results and to add new drugs to existing ones.

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Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

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