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Investigation of the Effect of *Cuscuta spp*. Extract on Cell Viability

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Abstract

Cancer is becoming an increasingly serious health problem worldwide. While various treatment approaches exist for diseases like colorectal cancer, they have not yet been found to be fully effective. Therefore, the development of new anti-cancer drugs is crucial. Cuscuta is a plant used in functional foods and for traditional medicinal purposes in various cultures, including Ayurveda and its medicinal traditions, which are widespread throughout the world. In preclinical studies, Cuscuta spp. have demonstrated anticytotoxic and cytoprotective properties and potential anticancer effects, particularly in various cell lines and animal models. Fibroblasts are scientifically proven to be the primary normal cell type of connective tissue, typically mesenchymal in origin. This is represented by the laboratory L929 mouse fibroblast cell line. Colorectal cancer is the third most common cancer worldwide and the third most common cause of cancer-related death. Our study aimed to determine the cytotoxic effects of *Cuscuta spp* extract, known to have medicinal effects in various diseases in public health, on the L929 mouse fibroblast cell line and the HT-29 human colorectal adenocarcinoma cell line. For cytotoxic analysis, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] enzymatic test was preferred. In vitro testing using *Cuscuta spp* extract revealed decreased viability in L929 fibroblast cell line and HT-29 human colorectal adenocarcinoma cells exposed to *Cuscuta spp* plant extract for 24 and 48 hours. The plant extract showed more cytotoxic effects on fibroblast cells, a normal cell line. In conclusion, this study demonstrates that *Cuscuta spp*. extract exhibits high cytotoxicity on normal cells and has limited effects on cancer cells. Further studies are needed for its pharmacological use.

Keywords: *Coscuta* spp, HT-29 cell, L929 fibroblast cell, Colorectal cancer, Cytotoxicity

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INTRODUCTION

Cuscuta spp, commonly known as *Cuscuta* herb or by other local names such as tuberculosis, devil's hair, gin's hair, and red herb, is a genus containing over 201 species of yellow, orange, or red (rarely green) parasitic plants. Previously considered the sole genus of the Cuscutaceae family, *Cuscuta spp* is now recognized as belonging to the morning glory family, Convolvulaceae, based on studies by the Angiosperm Phylogeny Group (Wu et al., 2022). This reclassification reflects a more thorough understanding of the plant's evolutionary relationships. The genus is found in temperate and tropical regions, worldwide, with the greatest species diversity occurring in subtropical and tropical regions, becoming rare in cool temperate climates (Welsh et al., 2010). This genus is unique in its parasitic lifestyle, distinguishing it from many other medicinal plants. It is characterized by its slender, often leafless stems with leaves reduced to small scales and very low chlorophyll levels, making it highly dependent on host plants for nutrition. Dodder has a large host population, generally preferring dicotyledons (Kumar and Amir., 2021). Dodder (*Cuscuta epithymum*) is a whitish-yellow fully parasitic plant that lacks chlorophyll, leaves, and roots and therefore must obtain its nutrients from other plants. The genus is found in temperate and tropical regions of the world; the greatest species diversity occurs in subtropical and tropical regions, becoming rare in cool temperate climates, with only four species native to Northern Europe. This wide distribution contributes to its widespread traditional use in different cultures (Noureen et al., 2019).

Cuscuta spp. species have a long and established history of use as key ingredients in functional foods and traditional medicinal systems across various cultures, including traditional Chinese medicine (where C. chinensis seeds are known as túsīzǐ), Ayurveda, and Himalayan regional medicinal traditions. The plant's widespread traditional application underscores its perceived therapeutic value (Donnapee et al., 2014). This genus is richly used as a medicinal plant in many regions of the world, and numerous biologically active compounds with therapeutic value have been isolated and demonstrated. These include the treatment of psychological problems, reproductive problems, scabies, certain tumors, viral jaundice, chronic ulcers, breast pain, osteoporosis, kidney inflammation, edema relief, gastric ulcers, and mghena (Ahmadi and Dehaghi., 2022).

Numerous *Cuscuta* species have been identified to possess various components effective against various diseases. Bioactive compound analyses in *Cuscuta* species have confirmed the presence of physiologically active components such as flavonoids, alkaloids, lignans, saponins, phenolics, tannins, and fatty acids through phytochemical studies. The antibacterial, antioxidant, antiostioporotic, hepatoprotective, anti-inflammatory, anticancer, antipyretic, antihypertensive, analgesic, anti-hair loss, and antisteriogenic properties of these plants have been demonstrated through pharmacological research and traditional practices (Noureen et al., 2019). *Cuscuta* species contain a wide range of chemical components, varying depending on the host species they invade. Among these, abundant flavonoids, phenolic compounds, some steroids, volatile oils, lignans, active polysaccharide compounds, fatty acids, and various alkaloids have been identified (Donnapee et al., 2014). Flavonoids constitute 3% of the overall phytochemical composition of *Cuscuta*. Primary flavonoid compounds such as kaempferol, quercetin, hyperoside, astragalin, and ligands in *Cuscuta* are important for their therapeutic effects on various diseases (Williamson et al., 2005).

Traditionally, *Cuscuta* has been prescribed as a general tonic, laxative, sudorific, anthelmintic, and diuretic. It has been used for a wide variety of ailments, including skin conditions (e.g., pruritus, scabies, eczema, chronic ulcers), painful inflammations, gout, joint pain, rheumatism, headache, fever, cough, bronchitis, respiratory disorders, gastrointestinal problems (e.g., diarrhea, stomach ache, constipation), liver and kidney health (e.g., jaundice, kidney problems), and various neurological disorders (e.g., seizures, melancholy, amnesia, epilepsy). Preclinical studies indicate that *Cuscuta spp* exhibits notable anticytotoxic and cytoprotective properties, including potent antioxidant, hepatoprotective, and neuroprotective effects, suggesting a differential effect on malignant cells versus healthy cells. *Cuscuta spp* also reveals significant anticancer potential in various cancer cell lines (e.g. pancreatic, leukemic, breast, prostate, colorectal) and animal models, primarily through inducing apoptosis, regulating the cell cycle and modulating gene expression (Mishra et al., 2022).

Fibroblasts are the fundamental cell type of connective tissue, typically of mesenchymal origin, and are widely distributed throughout the body for various repair functions. Within their tissue, they are rich in extracellular matrix (ECM) that supports a wide variety of essential organ functions, such as impaired soft tissue healing, secretion of cytokines such as growth factors, stem cell signaling, hemostasis, resistance to blunt and excessive injury in the skin, or organ-wide stretch and elastic recoil in the healthy, breathing lung (Boraldi et al., 2024). Fibroblasts are frequently used in basic cellular and molecular biology research due to their exceptional growth potential across multiple passages, high environmental flexibility, and the potential to be reprogrammed to become pluripotent stem cells for various regenerative medicine applications (Chehelgerdi et al., 2023).

Colorectal cancer is the third most common cancer worldwide and the third most common cause of cancer-related death. Nodal involvement is a key factor affecting survival, with 5-year survival ranging from 90% in early disease to 10% in metastatic disease (Menon and Cagir., 2025). Despite ongoing research, colorectal cancer remains one of the gastrointestinal neoplasms with the highest morbidity and mortality rates worldwide. HT-29 is an in vitro adherent cell line with an epithelial-like morphology. One of the most significant contributions of the HT-29 cell line is its ability to provide a dual-identity model that can be used in both cancer biology and normal gastrointestinal physiology. HT-29 colon cancer cells were selected as a model because colon cancer is the third most lethal cancer type worldwide (Neto et al., 2023).

The significant increase in cancer over the past thirteen decades has driven researchers to develop new treatment methods. The application of these methods has also increased interest in natural products as a way to mitigate the adverse chemical effects associated with traditional medicines. Previous studies have highlighted plants with significant antimicrobial and antioxidant properties, particularly regarding their role in strengthening the immune system. Comprehensive analysis is necessary to determine the effective doses of these plants and ensure their appropriate use for therapeutic purposes (Ali Abdalla et al., 2022).

This study aims to screen the cytotoxic potential of *Cuscuta spp*. herbal extract used in traditional medicine for various disorders, including diseases such as cancer.

MATERIALS AND METHODS

Preparation of *Cuscuta spp.* plant material

Cuscuta spp was obtained from a field on the Mardin Artuklu University campus. *Cuscuta spp.* were described botanically and morphologically by Mardin Artuklu University Plants Department plant taxonomist Dr. Fatma Mungan Kılıç. (Figure 1). The drying process was carried out in a laboratory environment away from sunlight. After drying, *Cuscuta* was ground and weighed to 100 g. The weighed sample was placed in a Soxhlet cartridge, and 300 mL of Merck's 997% pure methanol was added to one of the flasks to obtain the extract. The extract was filtered through Whatman filter paper. The solvent was evaporated using a rotary evaporator. The obtained plant extract was stored in a deep freezer at -22°C in Mardin Artuklu University Laboratory for 50 days. (Canpolat et al., 2024, Aktepe et al., 2023).

Figure 1. *Cuscuta spp* parasitic plant

Cell Culture Preparation

Our study used the frozen L929 mouse fibroblast cell line and the HT-29 human colorectal adenocarcinoma cell lines obtained from the American Type Culture Collection (ATCC). The L929 mouse fibroblast cell line is representative of normal cells, and the HT-29 cell line is a widely used cancer cell line derived from human colorectal adenocarcinoma. This cell line is particularly valuable in cancer research due to its well-differentiated structure and genetic characteristics. HT-29 cells harbor the p53-R273H contact mutant, which can disrupt the normal cell response to DNA damage. The cell lines were incubated in DMEM (Dulbecco's Modified Eagle Medium) supplemented with FBS (fetal bovine serum) and antibiotics (penicillin and streptomycin). For cellular proliferation, cell cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 (Aktepe et al., 2025).

Cytotoxic Activities with Cell Culture and MTT Assay

To investigate cell viability and the IC50 value of *Cuscuta spp*., the MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] enzymatic assay was used (Re et al., 1999). The MTT assay was used to determine the toxic effects of *Cuscuta spp* parasitic plant extracts at different time intervals. Cytotoxic activity on cells was tested with the L929 fibroblast cell line and the HT-29 human colorectal adenocarcinoma cell lines obtained from the American Type Culture Collection (ATCC) at the Dicle University Cell Culture Laboratory. The cell lines used in our study were incubated in T75 culture flasks in DMEM (Gibco 41965039, England) containing 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin at 37°C and 5% CO_2. Briefly, the cell lines were seeded in 96-well plates at a density of 1 × 104 cells per well and incubated for 24 hours for cell adhesion. When the cell number reached 80–90% of the sufficient number, they were removed from the flasks and their numbers were determined using the hemocytometric method (Baran et al., 2025). The counted cells were seeded in 96-well plates containing 90 µL of medium per well, with L929 fibroblast cell line and HT-29 human colorectal adenocarcinoma (8x103) cells per well and two different time treatments (24 h and 48 h) in triplicate. The next day, *Cuscuta spp* plant extract was prepared at various concentrations (1.00, 25.00, 50.00, 100.00 µg/mL) and applied to the culture plates; ultrapure water was applied to the cells in the control group. An MTT assay was performed to determine changes in cell viability 24 and 48 h after the treatment. 10 µL of the prepared MTT (5 mg/mL) solution was added to each well, and the cells were incubated for 3 h at 37°C in a humidified atmosphere containing 5% CO_2. After 3 h, the medium was removed from the wells and replaced with 100 µL of DMSO. Absorbances were read at 540 nm using a microplate reader (n = 8). Cell viability was estimated by the conversion of the tetrazolium salt to colored formazan crystals. Assays were repeated three times, and the results were averaged.

RESULTS AND DİSCUSSION

In vitro tests revealed a decrease in the viability of L929 fibroblast cell line and HT-29 human colorectal adenocarcinoma cells exposed to *Cuscuta spp* plant extract for 24 and 48 hours. This cytotoxic effect was observed in the L929 fibroblast cell line, with a 60% cell reduction after 24 hours and a 70% cell reduction after 48 hours. In HT-29 human colorectal adenocarcinoma cells, a cytotoxic effect of 10% after 24 hours and a 20% cell reduction after 48 hours was observed (Figure 2,3).

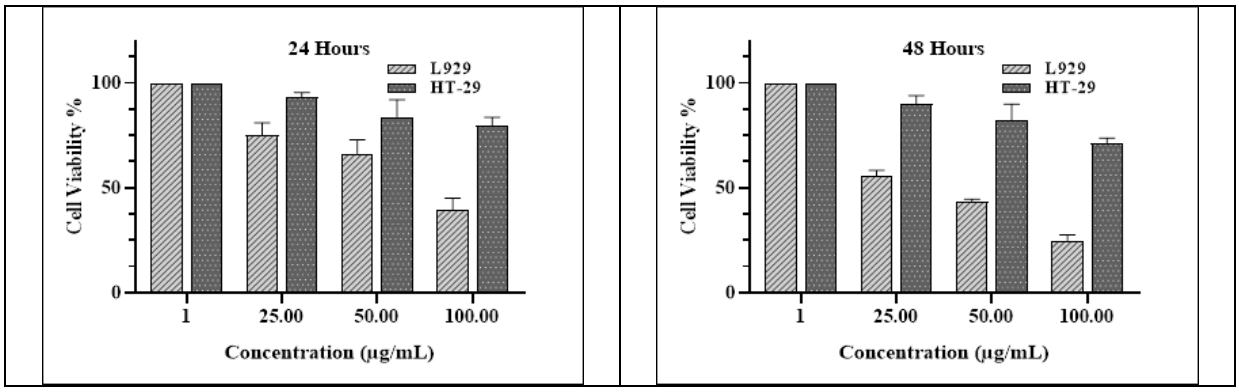

Figure 2: *Cuscuta* spp plant extract was treated in L929 and HT-29 cell lines exposed for 24 h and 48 h. Cytotoxicity was determined using MTT assays and results were expressed as percentages of cytotoxicity compared to the untreated control.

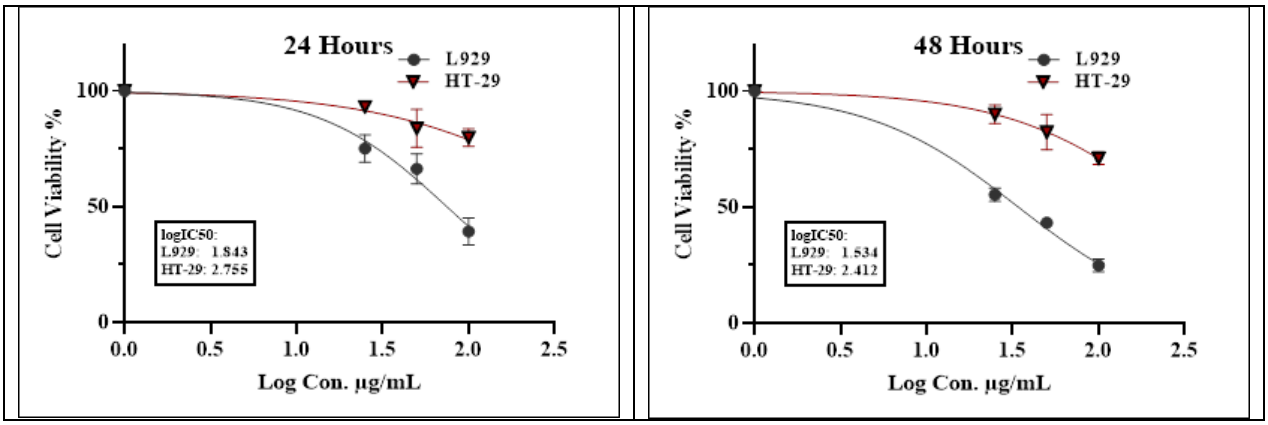

Figure 3: Dose-dependent effective concentration graph of L929 and HT-29 cells exposed to *Cuscuta spp* plant extract for 24 hours and 48 hours.

According to the laboratory results obtained in this study, the *Cuscuta* spp. plant extract exhibited greater cytotoxic effects on normal fibroblast cells (L929) and less cytotoxic effects on cancer cells (HT-29). In other words, the plant extract caused more damage to healthy cells than to cancer cells. As is known, *Cuscuta spp*. is a parasitic plant, and its extract contents are not very similar. The physiological contents of a plant and the bioactive compounds of the plants it parasitizes can vary significantly, leading to differences in antioxidant and cytotoxic properties (Lozanova et al., 2025). Our data indicate significant differences in the cytotoxic effects of *Cuscuta spp*. extract on L929 fibroblast cells compared to HT-29 cancer cells. Normal fibroblast cells (L929) showed a significant decrease in viability at both 24 hours (60% decrease) and 48 hours (70% decrease), while the decrease in cancerous HT-29 cells was relatively less pronounced (10% at 24 hours and 20% at 48 hours). The decrease in cell viability increased with increasing exposure time to the plant extract for both cell lines (from 24 to 48 hours), indicating that the effect of the extract is time-dependent. This suggests that the extract's components gradually disrupt intracellular processes, leading to cell death. However, the fact that only a 20% decrease in cell viability was observed in HT-29 cells after 48 hours suggests that this extract alone does not possess sufficient antitumor potential. Another possibility that comes to mind at this point is that HT-29 cells may have developed a resistance mechanism against the extract's cytotoxic effects. Other cytotoxicity studies conducted on the plant used in this study indicate that *Cuscuta species* possess biological activities, including cytotoxic potential. However, this does not necessarily mean that *Cuscuta* is harmful. Based on these findings, it is crucial that the dose, species, and extraction method be carefully determined, especially when using the plant for medicinal purposes. Some studies indicate that *Cuscuta* extracts exhibit cytotoxic effects on normal cells, such as L929 fibroblasts, similar to those on cancer cells. This suggests that *Cuscuta* species used in traditional medicine should be used with caution. One study found that *Cuscuta* epithymum extract exhibited similar cytotoxicity on normal fibroblasts and cervical cancer cells. Abedini et al.'s in vitro cytotoxicity assessment of *Cuscuta epithymum* extract on human fibroblasts and cervical cancer cells using the MTT method yielded similar results to ours. In other words, dodder extract showed more effective cytotoxicity against normal cells and relatively less cytotoxicity against cancer cells (Abedini et al., 2021). Selvi and Önal conducted a cytotoxicity analysis on the *Cuscuta* campestris species and conducted experiments on several cancer cell lines. Consequently, in this species, it exhibited a dose-dependent cytotoxic effect on cell viability starting at 1 mg/ml in MDA-MB-231 and RL-95-2 cell lines, and starting at 5 mg/ml in MCF-7, SH-SY5Y, and COLO 205 cell lines (Selvi and Önal 2024). These studies also indicate that the potential cytotoxic effects of *Cuscuta species* may vary depending on the plant species and the host plant on which it parasitizes. This suggests that the chemical composition of the plant can be affected by environmental factors.

In a study conducted by Alijaniha et al., they treated irradiated and non-irradiated *Cuscuta* extracts with HT-29 cell lines. The irradiated extracts were shown to exhibit more cytotoxic effects. In fact, this study can be said to support our study (Alijaniha et al., 2021).

In a study conducted by Ghazanfari et al., (2025), the cytotoxic effect of a traditional Cuscuta extract was investigated on human Burkitt lymphoma (Raji) and melanoma cell lines (SK-MEL-3) using the MTT method. Compared to the control group, Cuscuta extract was shown to have the highest cytotoxic effect on SK-MEL-3 and Raji cell lines, with a rate of 80% and 81%, respectively. According to a study by Dermani et al., C. chinensis reduced PC3 and MCF7 cell viability in a way that was dependent on both time and dose ($p<0.01$ to $p<0.001$). In comparison to the control group, cells treated with C. chinensis exhibited higher levels of the BAX/Bcl2 ratio, Caspase3, and PTEN gene expression. They showed that C. chinensis increased LDH activity ($p<0.01$ to $p<0.001$) and apoptosis ($p<0.001$). They demonstrated in this experiment that C. chinensis extract may cause PC3 and MCF7 cell lines to undergo apoptosis and limit growth. These tests indicated that the extract from C. chinensis had anticancer action against cancerous cells.

Cuscuta extracts have been documented as cytotoxic on the HT-29 cell line. One study showed that chloroform extracts from *C. chinensis* and *C. epithymum* significantly reduced the viability of HT-29 cells (Jafarian et al., 2014, Abedini et al., 2021). This effect was assessed using cell viability tests such as the MTT test for PC3 and MCF7 cells. Beyond this general observation, a link can be established between the specific compounds contributing to the effects (Nitthikan et al., 2024).

The primary reasons why herbal products and their derivatives are preferred in treatment are that they are compatible with the body's immune system and, when effective, help increase body resistance without harming healthy cells (Lopes et al., 2017). Anticancer drugs used in conventional treatments are classified as mitosis inhibitors, alkylating compounds, antimetabolites, cytostatic antibiotics, hormones and hormone antagonists, other cytostatics (asparaginase, cisplatin, and carboplatin), radioactive isotopes, interferons, and tyrosine kinase inhibitors (Pucci et al., 2019).

4. CONCLUSION

Plants, medicinal plants, and products derived from these plants, such as extracts, drugs, and so on, are widely used worldwide in the treatment of various diseases. However, scientific data on the biological effects and mechanisms of action of most extracts and bioactive compounds derived from medicinal plants are still insufficient. Therefore, it is important to scientifically demonstrate the medicinal effects of wild plants used for medicinal purposes, commonly known as dodder herb or tuberculosis herb, devil's hair herb, ginsacçı, and red herb. The data obtained indicate that the Cuscuta spp. extract studied failed to exhibit the desired high cytotoxic effect on cancer cells (HT-29) but instead caused significantly more damage to normal cells (L929). In light of these findings and the variable results reported in the literature for different Cuscuta species, careful determination of the dose, species, and extraction method is crucial when using the plant for medicinal purposes. A desirable characteristic of chemotherapy agents is their ability to preferentially target cancer cells over healthy cells. In contrast, this study observed the opposite. Consequently, additional research is needed to reduce the negative effects of this extract on normal cells or to increase its effectiveness against cancer cells. In conclusion, this study demonstrates that *Cuscuta spp*. The extract demonstrates high cytotoxicity on normal cells and limited effects on cancer cells. As such, its potential for clinical use is low, but further research could develop new treatment strategies by isolating the extract's beneficial components or using them in different combinations.

Compliance with Ethical Standards

Peer Review

This article has been reviewed by independent experts in the field using a rigorous double-blind peer review process.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

All authors contributed equally to the study design, data collection, analysis, and manuscript preparation.

Ethics Committee Approval

Ethical approval was not required for this study.

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