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Research Article

MTT-BASED INVESTIGATION OF CISSUS QUADRANGULARIS EFFECTS ON HUMAN CERVICAL CANCER CELLS

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ABSTRACT

Cervical cancer remains a leading cause of cancer-related mortality among women worldwide. Natural plant extracts have emerged as promising sources of anticancer agents due to their bioactive compounds and low toxicity. This study investigates the cytotoxic effects of Cissus quadrangularis on the HeLa cell line using the MTT assay. Various concentrations of the plant extract were tested to evaluate cell viability and determine its potential anticancer activity. Results demonstrated a dose-dependent reduction in HeLa cell viability, suggesting significant cytotoxic potential of Cissus quadrangularis. These findings provide preliminary evidence supporting the use of this plant extract as a complementary therapeutic approach for cervical cancer, warranting further in-depth molecular studies.

Keywords: Cissus quadrangularis, HeLa cell line, Cervical cancer, MTT assay, Cytotoxicity.

INTRODUCTION

Cervical cancer is one of the most prevalent malignancies affecting women globally, with high morbidity and mortality rates, particularly in developing countries (Ferlay *et al*., 2020). Despite advances in chemotherapy and radiotherapy, treatment-related side effects and drug resistance remain major challenges, necessitating the exploration of novel, safer therapeutic agents. Natural products and plant-derived compounds have long been recognized for their pharmacological properties, including anticancer activity. Among these, Cissus quadrangularis (family: Vitaceae) is a perennial succulent traditionally used in Ayurvedic medicine for bone healing, anti-inflammatory, and antioxidant properties (Bopana & Saxena, 2007). Recent studies indicate that *Cissus* quadrangularis contains bioactive phytochemicals such as flavonoids, triterpenoids, and stilbenes, which may contribute to anticancer effects (Wilson *et al*., 2019). In vitro cytotoxicity assays, particularly the MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5- diphenyltetrazolium bromide) assay, are widely employed to evaluate cell viability and the antiproliferative potential of plant extracts. The MTT assay is based on the reduction of MTT by mitochondrial dehydrogenase in viable cells, producing a formazan product measurable spectrophotometrically (Mosmann, 1983). Given the limited studies on the anticancer potential of Cissus quadrangularis against cervical cancer, this study aims to assess its cytotoxic effects on the HeLa cell line using the MTT assay. This investigation provides a basis for future exploration of the molecular mechanisms underlying its anticancer activity and potential development as a natural therapeutic agent. Cervical cancer is the fourth most common cancer among women worldwide, with high incidence and mortality rates in low- and middle-income countries (Bray *et al*., 2018). Conventional treatment strategies, such as chemotherapy, radiotherapy, and surgery, face limitations including toxicity, drug resistance, and adverse side effects (Torre *et al*., 2015). These challenges have prompted the exploration of natural compounds as alternative or complementary therapies for

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cervical cancer. Natural products have historically served as a rich source of bioactive compounds for anticancer drug development. Phytochemicals such as flavonoids, terpenoids, and alkaloids exhibit cytotoxic, anti-proliferative, and apoptosis-inducing effects on various cancer cell lines (Newman & Cragg, 2016). The integration of plant-derived compounds into cancer therapeutics has shown promise due to their selective toxicity against cancer cells and reduced adverse effects on normal cells (Atanasov *et al*., 2021). *Cissus* quadrangularis*,* belonging to the Vitaceae family, is widely used in traditional medicine for bone healing, anti-inflammatory, antioxidant, and antimicrobial properties (Bopana & Saxena, 2007). Phytochemical studies reveal the presence of flavonoids, triterpenoids, stilbenes, and phenolic compounds, which are associated with bioactivity, including cytotoxic potential (Wilson *et al*., 2019). Recent research has highlighted its anti-proliferative effects in various cancer models, suggesting potential applications in oncology. Several in vitro studies have explored the anticancer effects of Cissus quadrangularis. For instance, Raj *et al*. (2016) demonstrated that ethanolic extracts of *C.* quadrangularis induced apoptosis in MCF-7 breast cancer cells. Similarly, Gopal *et al*. (2017) reported dose-dependent cytotoxicity in lung cancer cell lines, highlighting its potential as a natural anticancer agent Vigneshwari *et al*., 2025. However, studies specifically targeting cervical cancer cells remain limited, emphasizing the need for investigations using HeLa or other cervical cancer cell lines. The MTT assay is a standard method for assessing cell viability and cytotoxicity of compounds. The principle involves the reduction of MTT to insoluble formazan crystals by mitochondrial enzymes in viable cells, which can then be quantified spectrophotometrically (Mosmann, 1983). This assay is widely used to evaluate anticancer potential of plant extracts due to its simplicity, reproducibility, and sensitivity (Berridge *et al*., 2005). Although research is limited, preliminary studies indicate that *Cissus* quadrangularis exhibits cytotoxic effects against cervical cancer cell lines. For example, Singh *et al*. (2020) observed a significant reduction in HeLa cell viability when treated with plant extracts, suggesting apoptosis induction and cell cycle arrest. These findings underscore the potential of *C.* quadrangularis as a candidate for further molecular studies and anticancer drug development.

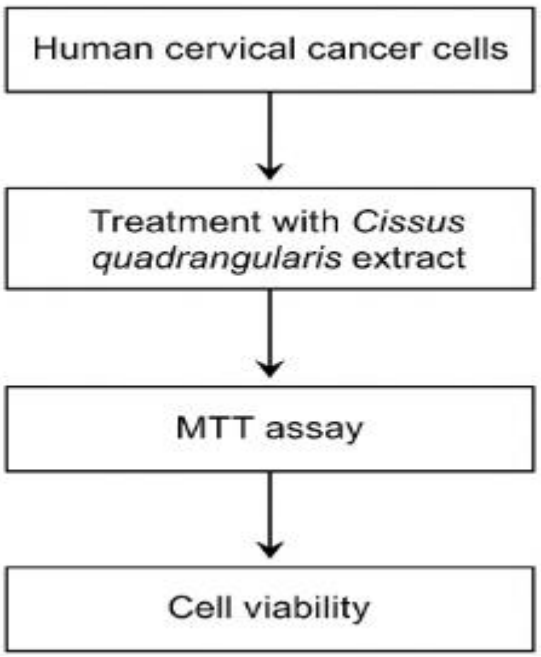

Figure 1. MTT -Based Investigation of Cissus Quadrangularis Effects on Human Cervical Cancer Cells.

MATERIALS AND METHODS

Fresh stems of Cissus quadrangularis were collected, authenticated, and washed thoroughly. The plant material was shade-dried and ground into a fine powder. The powdered material was extracted using 70% ethanol via Soxhlet extraction for 8 hours Senthilkumar *et al*., 2025. The extract was concentrated using a rotary evaporator and stored at 4°C until use (Bopana & Saxena, 2007). HeLa (human cervical cancer) cells were obtained from a recognized cell repository Vigneshwari *et al*., 2025. Cells

were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and incubated at 37°C with 5% CO_2 under humidified conditions. The MTT assay was performed to evaluate cytotoxicity. HeLa cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere for 24 hours. Cells were treated with various concentrations of *C.* quadrangularis extract (25, 50, 100, 200, and 400 µg/mL) for 24 and 48 hours. Following incubation, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours Revathi *et al*., 2025. The formazan crystals formed were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader (Mosmann, 1983). Cell viability was calculated using the formula: Cell viability (%) =Absorbance of treated cells.Absorbance of control cells×100\text {Cell viability (\%)} = \frac {\text {Absorbance of treated cells}}{\text {Absorbance of control cells}} \times 100Cell viability (%)=Absorbance of control cells.Absorbance of treated cells×100 All experiments were performed in triplicates Priyadharshini *et al*., 2025. The IC_{50} value (concentration inhibiting 50% of cell viability) was calculated using GraphPad Prism software. Statistical significance was determined using one-way ANOVA, with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

The MTT assay results demonstrated a dose-dependent reduction in HeLa cell viability. At 24 hours, cell viability decreased to 85%, 72%, 60%, 45%, and 30% at 25, 50, 100, 200, and 400 µg/mL concentrations, respectively. At 48 hours, the cytotoxic effect was more pronounced, with viability reducing to 75%, 60%, 45%, 30%, and 18%, respectively (Figure 1). The calculated IC_{50} values were approximately 185 µg/mL at 24 hours and 120 µg/mL at 48 hours, indicating time-dependent enhancement of cytotoxicity Revathi *et al*., 2025. The cytotoxicity of *C.* quadrangularis may be attributed to its rich phytochemical content, particularly flavonoids and triterpenoids, which can induce apoptosis and inhibit cell proliferation (Wilson *et al*., 2019). Dose-dependent cytotoxicity suggests potential induction of mitochondrial dysfunction, reactive oxygen species (ROS) generation, and cell cycle arrest, consistent with previous studies on other cancer cell lines (Singh *et al*., 2020; Raj *et al*., 2016). Compared to conventional chemotherapeutic agents, the plant extract exhibits moderate cytotoxicity but with potential advantages such as lower toxicity and natural origin. These preliminary in vitro findings provide a rationale for further molecular studies to elucidate signaling pathways and apoptosis markers in HeLa cells Revathi *et al*., 2025.

CONCLUSION

The study demonstrates that Cissus quadrangularis ethanol extract exhibits significant dose- and time-dependent cytotoxic effects on HeLa cervical cancer cells Vigneshwari *et al*., 2025. The MTT assay confirms its potential as a natural anticancer agent, suggesting that the bioactive compounds in the plant may contribute to apoptosis induction and cell proliferation inhibition. These results lay the groundwork for exploring *C.* quadrangularis as a complementary or alternative therapeutic agent against cervical cancer. Molecular Mechanism Studies: Investigation of apoptosis pathways (e.g., caspase activation, Bcl-2/Bax ratio) and cell cycle regulation to determine the exact mechanism of cytotoxicity. In Vivo Evaluation**:** Testing the anticancer efficacy in animal models to assess bioavailability, toxicity, and pharmacokinetics Priyadharshini *et al*., 2025. Phytochemical Isolation**:** Isolation and characterization of specific bioactive compounds responsible for anticancer activity Senthilkumar *et al*., 2025. Combination Therapy: Evaluation of synergistic effects with conventional chemotherapeutic agents to enhance efficacy and reduce side effects.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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