



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

¹*Ramesh M, ²Subha SC, ³Vijai Krishna V, ⁴Madhumitha N and ⁵Linisha N M

¹*PERI Institute of Technology, Chennai - 48, Tamil Nadu, India

²PERI College of Arts and Science, Chennai -48, Tamil Nadu, India

³PERI College of Physiotherapy, Chennai -48, Tamil Nadu, India

⁴PERI College of Pharmacy, Chennai -48, Tamil Nadu, India

⁵PERI College of Nursing, Chennai -48, Tamil Nadu, India

Article History: Received 27th September 2025; Accepted 26th November 2025; Published 1st December 2025

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

*Corresponding Author: Ramesh M, PERI Institute of Technology, Chennai - 48, Tamil Nadu, India Email: publications@peri.ac.in.

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.

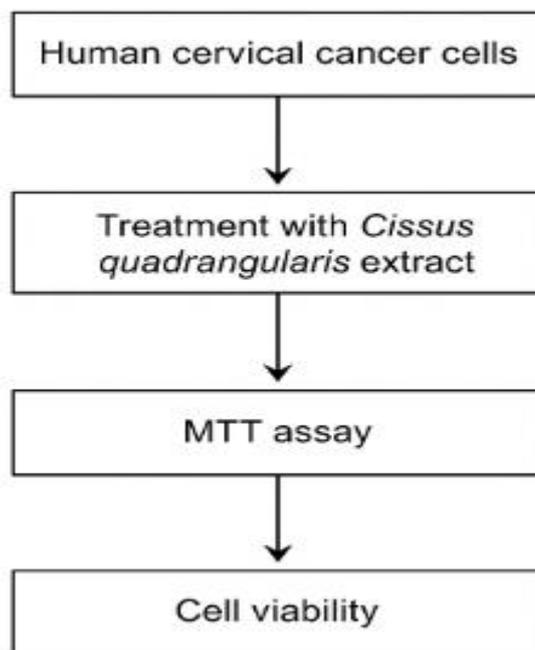


Figure 1. MTT -Based Investigation of *Cissus Quadrangularis* Effects on Human Cervical Cancer Cells.

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% CO₂ under humidified conditions. The MTT assay was performed to evaluate cytotoxicity. HeLa cells were seeded in 96-well plates at a density of 1 × 10⁴ 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. Cell viability (%) = Absorbance of control cells / Absorbance of treated cells × 100. All experiments were performed in triplicates Priyadarshini *et al.*, 2025. The IC₅₀ 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 IC₅₀ 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 Priyadarshini *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.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Zoology, Madras University for the facilities provided carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

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

REFERENCES

- Bhujade, A., Gupta, G., Talmale, S., Das, S. K., & Patil, M. B. (2013). Induction of apoptosis in A431 skin cancer cells by *Cissus quadrangularis* Linn stem extract by altering Bax–Bcl-2 ratio, release of cytochrome-c from mitochondria and PARP cleavage. *Food & Function*, 4(2), 338–346. <https://doi.org/10.1039/C2FO30167A>
- Berridge, M. V., Herst, P. M., & Tan, A. S. (2005). Tetrazolium dyes as tools in cell biology: New insights

- into their cellular reduction. *Biotechnology Annual Review*, 11, 127–152.
- Devasena, B., Kiran Kumar, S., Anitha, W., Balaji, B., & Mahalakshmi, J. (2005). Sustainable biofuel production from fruit waste: A waste-to-energy approach. *The Bioscan*, 20(2), S2, 606–609.
- Dhanasekaran, S., Rasmi, R., Subramanian, P., & Nachiappan, V. (2020). Phytochemical characteristics of aerial part of *Cissus quadrangularis* (L.) and its in-vitro inhibitory activity against leukemic cells and antioxidant properties. *Saudi Journal of Biological Sciences*, 27(5), 1302–1309. <https://doi.org/10.1016/j.sjbs.2020.01.005>
- Dwivedi, A., Seethalakshmi, I., & Sharmila, D. (2013). Anticancer properties of *Cissus quadrangularis*. *Journal of Chemical and Pharmaceutical Research*, 5(5), 135–139.
- Sheikh, S., Siddiqui, S., Dhasmana, A., Safia, Haque, E., Kamil, M., Lohani, M., Arshad, M., & Mir, S. S. (2015). *Cissus quadrangularis* Linn stem ethanolic extract liberates reactive oxygen species and induces mitochondria mediated apoptosis in KB oral epidermoid cancer cells. *Pharmacognosy Magazine*, 11(44 Suppl 2), S365–S374. <https://doi.org/10.4103/0973-1296.168972>
- Kontham, G. R., Vutukuru, G. V. K., Chepuri, K., Chittepu, P., Vadakavila, G., Addepally, U., & Vutukuru, S. S. (2024). In vitro assessment of antioxidant and cytotoxic activities of *Cissus quadrangularis* using HeLa cells. *Asian Journal of Biological and Life Sciences*, 13(3), 760–769.
- Mohandoss, K., Vijayan, V., & Hemalatha, S. (2024). *Cissus quadrangularis* phytosomes' bioactivity assessment: Cytotoxic, anti-inflammatory and antioxidant properties. *Asian Journal of Biological and Life Sciences*, 13(3), 714–722. <https://doi.org/10.5530/ajbls.2024.13.87>
- Nafisa Farheen, S., Sangeetha, E. S. S., Devasena, B., Ashwini, L., & Geetha, N. B. (2025). Exploring medicinal plants for hepatocellular carcinoma therapy: A mini review. *The Bioscan*, 20(2), S2, 590–592.
- Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products*, 79(3), 629–661.
- Payani, S., & Bhaskar, M. (2023). A study on antimicrobial and anticancer properties of *Cissus quadrangularis* using A549 lung cancer cell line. *Cancer Treatment and Research Communications*, 36, Article 100732. <https://doi.org/10.1016/j.ctarc.2023.100732>
- Prema, R., Sathish Sekar, D., Chandra Sekhar, K., & Jeevanandham, S. (2012). In vitro cytotoxicity study on combined plant extracts (*Cissus quadrangularis* & *Aegle marmelos*) against HT-29 colon cancer cells. *European Journal of Experimental Biology*, 2(4), 882–888.
- Zhao, X., Wang, Y., Zhang, Z., Velu, P., & Liu, R. (2024). In-vitro antioxidant, in-vitro and in-silico ovarian anticancer activity (ovarian cancer cells-PA1) and phytochemical analysis of *Cissus quadrangularis* L. ethanolic extract. *Combinatorial Chemistry & High Throughput Screening*, 27(10), 1504–1512. <https://doi.org/10.2174/0113862073255558230926114444>
- Senthil Kumar, K. S., Senthilkumar, G. P., Lavanya, R., Linisha, N. M., & Paranthaman. (2025). Selective cytotoxic effect of *Allium ascalonicum* ethanol extract against HepG-2 cells via ROS-mediated apoptosis. *The Bioscan*, 20(3), S.I(3), 980–986.
- Senthil Kumar, K. S., Senthilkumar, G. P., Lavanya, R., Linisha, N. M., & Sudha, M. (2025). Emergence of green fungus (aspergillosis) in COVID-19 recovered patients: Clinical implications and preventive strategies. *The Bioscan*, 20(3), S.I(3), 987–991.
- Sheikh, S., Dhasmana, A., Siddiqui, S., Safia, Haque, E., Kamil, M., Lohani, M., Arshad, M., & Mir, S. S. (2015). *Anticancer activity of Cissus quadrangularis: An in vitro 2D model-based st*
- Singh, V., Patel, R., & Tiwari, P. (2020). Study on cytotoxic and apoptotic effects of *Cissus quadrangularis* extract on HeLa cancer cells. *Pharmacognosy Research*, 12(3), 220–227.
- Suresh, P., Xavier, A. S., Karthik, V. P., & Punnaigai, K. (2019). Anticancer activity of *Cissus quadrangularis* L. methanolic extract against MG63 human osteosarcoma cells—In vitro evaluation. *Biomedical and Pharmacology Journal*, 12(2), 975–980.

