

# Anticancer Activity of *Cissus Quadrangularis*

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**Abstract:** Validation of the useful medicinal properties of various indigenous plants has gained immense importance as they can be used as an alternative source of lead compounds in pharmacological industries to tackle many modern day problems such as drug resistance in microorganisms and non-specificity in chemotherapeutic agents. *Cissus quadrangularis* Linn. is one such plant which has been widely used for medical purposes in traditional systems of medicine. Hence this work seeks to validate the anticancer property of methanolic and ethanolic extracts of the plant against HeLa cell line. The IC50 concentration was recorded at 62.5 ug/ml for methanolic extract and 125ug/ml for ethanolic extract. The results clearly revealed that the methanolic extract of *Cissus quadrangularis* was more potent on HeLa cell line when compared to ethanolic extract.

**Index Terms:** HeLa Cell line, Ethanol Extract, Methanol Extract.

## I. INTRODUCTION

One of the major health issues which plague the developed and developing countries alike is cancer. According to statistics, there are 2.5 million people affected by cancer of which 8,00,000 are new cancer cases and this disease is known to be the cause of the death of 5,50,000 people each year (Sheik et al, 2015). Modalities of treatment to tackle cancer include chemotherapy, radiation and surgery, which are given as individual therapy or in combination. Due to the various drawbacks associated with these treatments, a search for alternate, safe and cost efficient method continues.

Plants are great source of various bioactive compounds which make them candidates for the various drug Research. Many medical plants have been used in various traditional medical practices from time immemorial. Plant derivatives and secondary metabolites are enriched with multiple biological properties play a vital role in curing numerous lethal diseases (subramaniyam Deepika and Immanuel Selvaraj, 2016). Hence the scientific study on the extraction of active molecules and the evaluation of its anticancer potential is the need of the hour.

*Cissus quadrangularis* Linn. is one such plant which has been widely used for medical purpose. It is a cactus like jointed climber belonging to the family *Vitaceae*. It is called Pirandai in Tamil. This perennial plant is native to India or Sri Lanka, Its stem is quadrangular in shape having four winged internodes constricted at nodes (The Ayurvedic pharmacopoeia of India). The various bioactive compounds like flavanoids and indanes, polysterol and kerosteroid in *Cissus quadrangularis* Linn, made this as one of the important medicinal plants and these bioactive compounds

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have been used in piles, bone fracture, pain in joints, swelling, scurvy, gout and also acts as an antioxidant, antimicrobial, anti-inflammatory and anticancer compounds (Sadhana et al., 2018). Since this plant with novel bioactive compounds has been proven to show promising therapeutic and pharmacological applications, the present study was undertaken to find out the potential of *Cissus quadrangularis* Linn on the survival of cervical cancer cell line – HeLa.

## II. MATERIALS AND METHODS

*Cissus quadrangularis* Linn. plant was collected from the garden and the stand was legitimated by Prof P. Jayaraman, Plant Anatomy Research Centre, Chennai. The samples were shade dried and powdered. Twenty five gram of powdered sample was removed with 250 ml of methanol and ethanol separately using soxhlet apparatus for 10hrs. The extract was concentrated using rotary evaporator under reduced pressure at 50 °C. The residue was made to a concentration of 100mg/ml and stored in refrigerator for further use (Parang et al., 2013).

### *In vitro* anticancer Activity

HeLa cell line was attained from National centre for cell sciences Pune (NCCS). The cells were preserved in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a dampen ambience of 50 µg/ml CO<sub>2</sub> at 37 °C.

*In-vitro* assay of anticancer bustle of the methanol and ethanol extract of the stem of *Cissus quadrangularis* Linn. were studied against HeLa cell line using MTT Assay. Cells (1 × 10<sup>5</sup>/well) were seeded in 24-well plates and hatched in 37°C with 5% CO<sub>2</sub> condition. After attaining confluence, the cells were treated with various concentrations of the samples and further incubated for 24hrs. On completion all the wells were washed with DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was extra and hatched for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank (Mosmann, 1983). The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

## III. RESULTS

The result of the MTT Assay showing the % cell viability for the methanol and ethanol extracts of the stem of

*Cissus quadrangularis* on HeLa cell line is shown in Table 1 and 2. The lowest cell viability of 21.64% and 30.48% was obtained at a concentration of 1000µg/ml



## Anticancer Activity of Cissus Quadrangularis

of methanol and ethanol extract respectively, while it was highest at 73.65% and 74.45% at 7.8µg/ml of methanol and ethanol extract respectively. The IC<sub>50</sub> value which is the concentration of the sample at which 50% of the cancer cells are viable was found to be at the concentration of 65µg/ml for methanol extract and 125µg/ml for ethanol extract respectively.

Table.1 Anticancer effect of methanol extract of Cissus Quadrangularis extract on HeLa cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.350	21.64
2	500	1:1	0.435	26.90
3	250	1:2	0.544	33.64
4	125	1:4	0.664	41.06
5	62.5	1:8	0.783	48.42
6	31.2	1:16	0.912	56.40
7	15.6	1:32	1.053	65.12
8	7.8	1:64	1.191	73.65
9	Cell control	-	1.617	100

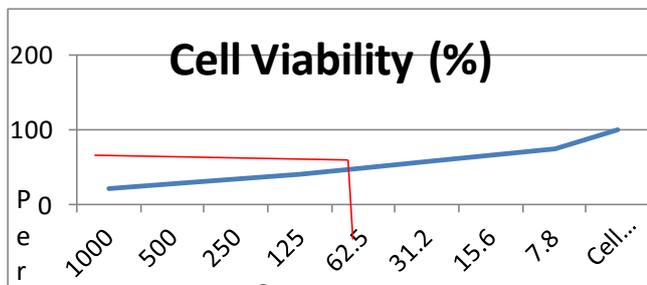


Fig.1 Cell Viability

Table.2 Anticancer effect of ethanol extract of Cissus quadrangularis on HeLa Cellline

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.493	30.48
2	500	1:1	0.584	36.11
3	250	1:2	0.672	41.55
4	125	1:4	0.788	48.73
5	62.5	1:8	0.879	54.35
6	31.2	1:16	0.989	61.16
7	15.6	1:32	1.115	68.95
8	7.8	1:64	1.204	74.45
9	Cell control	-	1.617	100

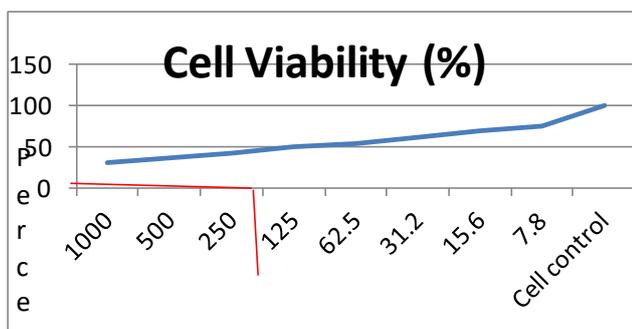


Fig.2 Anticancer effect of ethanol extract of Cissus quadrangularis extract on HeLa Cell line

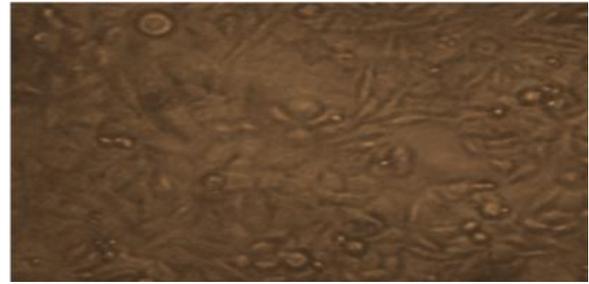


Fig.3 Toxicity- 1000µg/ml



Fig.4 Toxicity- 62.5µg/ml



Fig.5 Toxicity- 7.8µg/ml

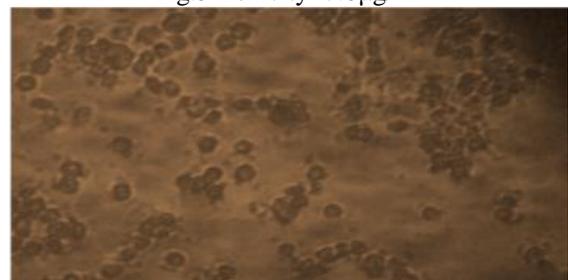


Fig.6 Anticancer effect of ethanol extract of Cissus quadrangularis extract on HeLa Cell line



Fig.7 Normal HeLa Cell line



Fig.8 Toxicity- 1000µg/ml



Fig.9 Toxicity- 125µg/ml



Fig.10 Toxicity- 7.58µg/ml

#### IV. DISCUSSION

*Cissus quadrangularis* Linn. has been shown to possess medicinal properties to cure a number of diseases. Various studies on the cytotoxic activity of *Cissusquadrangularis* on various cell lines has shown it to be useful for management of oral cancer, breast cancer, cervical cancer and siddha drug formulation consisting of *Cissusquadrangularis* has been used to treat various forms of cancer (Rajamaheswari, 2017).

The current investigation evaluates the cytotoxicity of methanol and ethanol extracts of *Cissusquadrangularis* on the HeLa cell lines. The methanol extract illustrated an IC<sub>50</sub> value at 62.5 µg/ml concentration while the ethanol extract showed an IC<sub>50</sub> value at 125 µg/ml concentration. Similar results have been carried out by Aayush Dwivedi *et al.*, (2013) who reported that the methanol and ethanol extracts of *Cissus quadrangularis* were found to exhibit anticancer activity in HeLa and Vero cell lines with IC<sub>50</sub> values of 62.5 µg/ml and 125 µg/ml respectively. Vijayalakshmi *et al.*, (2013) had stated that flavonoid fractions and ethanol extracts had shown anticancer activity in MCF7 cell line with the IC<sub>50</sub> values of 10 µg/mL and 40 µg/mL respectively. Sheikh *et al.* (2015) reported that *Cissus quadrangularis* suppresses the growth of HeLa cells without damaging the normal cells at the concentration of 200 µg/ml (IC<sub>50</sub>). It triggers the ROS liberation in cancer cells which

mediates the apoptosis and G1 phase cell cycle arrest.

Anticancer activity of the ethanolic extract of *Cissusquadrangularis* was observed in KB oral epidermoid carcinoma cells that resulted in changes in cell morphology like cell shrinkage, plasma membrane blebbing, loss of cell membrane asymmetry (Sheikh *et al.*, 2015). Nagani Krunal and Chanda Sumitra (2013) had shown that chloroform and ethanol extract of *Cissusquadrangularis* exhibited cytotoxicity of 80.60% and 85.40% at 1000 µl on Ehrlich Ascites Carcinoma cell line. Extract of *Cissusquadrangularis* in combination with the extract of *Aegle marmelos* showed cytotoxic activity against colon cancer HT29 cell lines.

#### V. CONCLUSION

In the present study, the methanol extracts showed an IC<sub>50</sub> at 62.5 µg/ml concentration while the ethanol extracts showed an IC<sub>50</sub> value at 125 µg/ml concentration. From these results it may be concluded that methanolic extract of *Cissusquadrangularis* has a more potent cytotoxic effect on the HeLa cell lines when compared to the ethanolic extract. Future studies may throw further light on the anti-cancerous activity of *Cissusquadrangularis*.

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