MTT Assay of Cissus Quadrangularison Hela Cell Line

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Abstract: Approval of the helpful restorative properties of different indigenous plants has increased monstrous significance as they can be utilized as an elective wellspring of lead mixes in pharmacological ventures to handle numerous advanced issues, for example, tranquilize obstruction in microorganisms and non-particularity in chemotherapeutic operators. Cissusquadrangularis Linn.is one such plant which has been generally utilized for therapeutic purposes in customary frameworks of prescription. Thus this work looks to approve the anticancer property of methanolic and ethanolic concentrates of the plant against HeLa cell line. The IC50 fixation was recorded at 62.5 ug/ml for methanolic separate and 125ug/ml for ethanolic remove. The outcomes unmistakably uncovered that the methanolic concentrate of Cissusquadrangularis was progressively powerful on HeLa cell line when contrasted with ethanolic extricate.

Keywords: HeLa Cell line, Ethanol Extract, Methanol Extract.

I. INTRODUCTION

One of the significant medical problems which plague the created and creating nations alike is malignant growth. As indicated by measurements, there are 2.5 million individuals influenced by malignancy of which 8,00,000are new malignant growth cases and this infection is known to be the reason for the demise of 5,50,000 individuals every year (Sheik et al, 2015). Modalities of treatment to handle malignant growth incorporate chemotheraphy, radiation and medical procedure, which are given as individual treatment or in blend. Because of the different disadvantages related with these medicines, a quest for substitute, safe and cost proficient strategy proceeds.

Plants are incredible wellspring of different bioactive mixes which make them possibility for the different medication Research. Numerous restorative plants have been utilized in different customary therapeutic practices from days of yore. Plant subsidiaries and auxiliary metabolites are advanced with various natural properties assume an indispensable job in restoring various deadly maladies (subramaniyamDeepika and Immanuel Selvaraj, 2016).Hence the logical examination on the extraction of dynamic particles and the assessment of its anticancer potential is the need of great importance.

Cissusquadrangularis Linn.is one such plant which has been generally utilized for medicinal reason. It is a desert plant like jointed climber having a place with the family Vitaceae. It is called Pirandai in Tamil. This lasting plant is local to India or Sri Lanka, Its stem is quadrangular fit as a fiddle having four winged internodes choked at hubs (The Ayurvedic pharmacopeia of India). The different bioactive mixes like flavanoids and indanes, polysterol and kerosteroid in *Cissusquadrangularis Linn* , made this as one of the significant restorative plantsand these bioactive mixes have been utilized in heaps ,bone crack ,torment in joints, swelling ,scurvy ,gout and furthermore goes about as an antioxidant,antimicrobial , hostile to – provocative and anticancer mixes (Sadhana et al ., 2018). Since this plant with novel bioactive mixes has been demonstrated to show promising restorative and pharmacological applications, the present examination was attempted to discover the capability of Cissusquadrangularis Linn on the survival of cervical disease cell line – HeLa.

II. MATERIALS AND METHODS

Cissusquadrangularis Linn. plant was gathered from the greenhouse and the plant was validated by Prof P.Jayaramen, Plant Anatomy Research Centre,Chennai.The tests were shade dried and powdered.Twenty five gram of powdered example was extricated with 250 ml of methanol and ethanol independently utilizing soxhlet mechanical assembly for 10hrs. The concentrate was concentrated utilizing turning evaporator under decreased weight at 50 C.The buildup was made to a convergence of 100mg/ml and put away in cooler for further use (Parang et al.,2013).

In vitro anticancer Activity

HeLacell line was gotten from National community for cell sciences Pune (NCCS). The cells were kept up in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified environment of 50 µg/ml CO2 at 37 °C.

In-vitro test of anticancer action of the methanol and ethanol concentrate of the stem of Cissusquadrangularis Linn. were contemplated against HeLa cell line utilizing MTT Assay. Cells (1 × 105/well) were seeded in 24-well plates and brooded in 370C with 5% CO2 condition. In the wake of accomplishing intersection, the cells were treated with different centralizations of the examples and further hatched for 24hrs. On fruition every one of 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 included and brooded for 4 hours. After hatching, 1ml of DMSO was included all the wells.The absorbance at 570nm was estimated with UV-Spectrophotometer utilizing DMSO as the clear (Mosmann, 1983). The % cell suitability was determined utilizing the accompanying recipe:

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% Cell viability = A570 of treated cells / A570 of control cells × 100

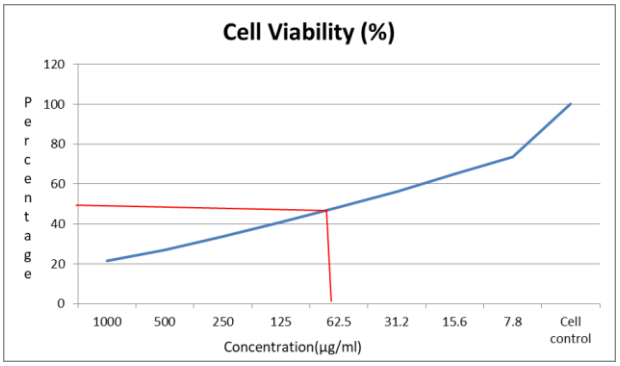
Results :

The consequence of the MTT Assay demonstrating the % cell feasibility for the methanol and ethanol concentrates of the stem of Cissusquadrangularis on Hela cell line is appeared in Table1and 2.The most minimal cell suitability of 21.64% and 30.48% was acquired at a centralization of 1000µg/ml of methanol and ethanol remove individually, while it was most elevated at 73.65% and 74.45% at 7.8µg/ml of methanol and ethanol extricate separately.

The IC50 esteem which is the grouping of the example at which half of the malignant growth cells are feasible was observed to be at the convergence of 65µg/ml for methanol separate and 125µg/ml for ethanol extract individually

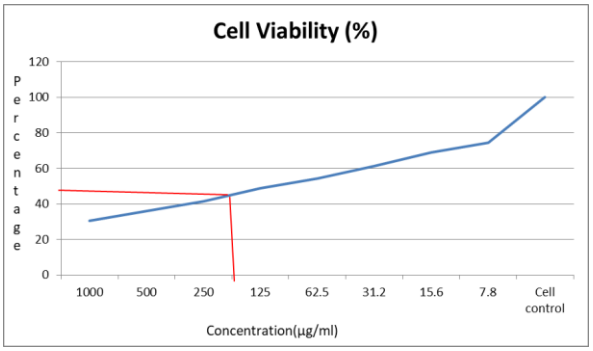
MTT assay of methanol extract of *Cissusquadrangularis* extract onHeLa cell line

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	CellViability (%)
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



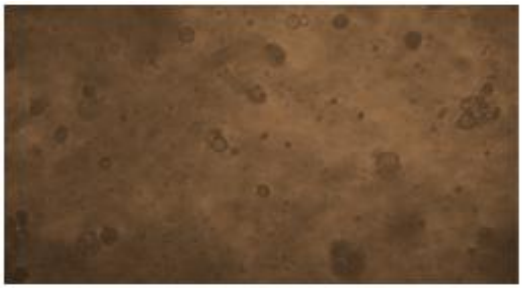
Anticancer effect of ethanol extract of *Cissusquadrangularis* on *HeLa Cell*line

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



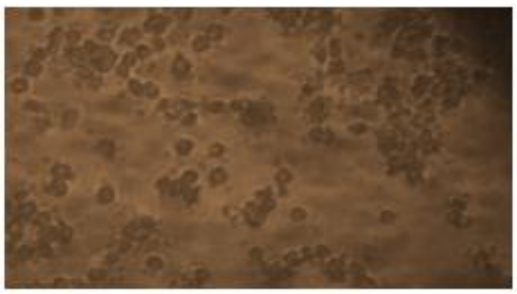
Anticancer effect of methanol extract of *Cissusquadrangularis* extract on*HeLaCell*line

Normal HeLa Cell line



Toxicity- 1000µg/ml

Toxicity- 62.5µg/ml

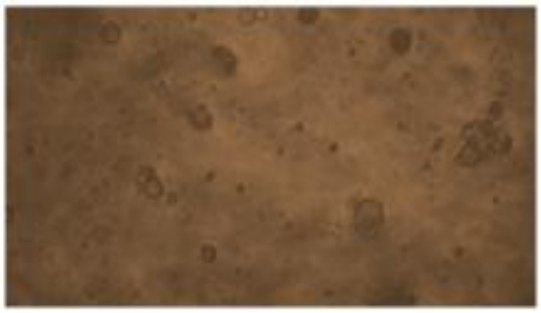


Toxicity- 7.8µg/ml

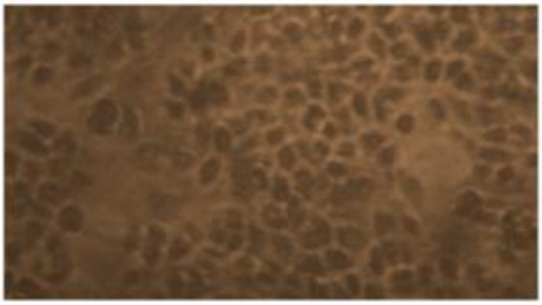
Anticancer effect of ethanol extract of *Cissusquadrangularis* extract on*HeLaCell*line

Normal HeLa Cell line

Toxicity- 1000µg/ml



Toxicity- 125µg/ml



Toxicity- 7.8µg/ml

III. DISCUSSION RESULTS

*Cissusquadrangularis*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 line has shown it to be useful for management of oral cancer, breast cancer, cervical cancerand siddha drug formulation consisting of *Cissusquadrangularis*has been used to treat various forms of cancer(Rajamaheswari,2017).

The present study evaluates the cytotoxicity of methanol and ethanol extracts of *Cissusquadrangularis*on the Hela cell lines. The methanol extracts showed an IC50 value at 62.5 µg/ml concentration while the ethanol extracts showed an IC50 value at 125µg/ml concentration. Similar results has been carried out by AayushDwivedi*et al.*,(2013)who reported that the methanol and ethanol extracts of *Cissusquadrangularis* was found to exhibiting anticancer activity in HeLa and Vero cell lines with IC50 values of 62.5 µg/ml and 125µg/ml respectively. Vijayalakshmi*et al.*, (2013) had stated that flavonoid fractions and ethanol extracts had shown anticancer activity in MCF7 cell line with the IC50 values of 10 µg/mL and 40 µg/mL respectively.

Sheikh et al (2015) reported that Cissusquadrangularis suppress the growth of HeLa cells without damaging the normal cells at the concentration of 200ug/ml (IC50).It triggers the ROS liberation in cancer cells which mediates the apoptosis and G1 phase cell cycle arrest.

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

IV. CONCLUSION

In the present study, the methanol extracts showed an IC50 at 62.5 µg/ml concentration while the ethanol extracts showed an IC50 value at 125µg/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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