



IN VITRO ANTI CANCER ACTIVITY OF VARIOUS FRACTIONS OF HYDRO ALCOHOLIC EXTRACT OF *DALBERGIA LATIFOLIA* ROXB.

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ABSTRACT

Dalbergia latifolia Roxb. (DLR) plant is majorly available in India, traditionally used for various diseases. Hence the aim of the present study is to compare the phytochemical investigation and *in vitro* anticancer activity of various fractions of hydro alcoholic extract of *Dalbergia latifolia* Roxb. against L6, EAC, MCF 7, HEP G2 and Hela cell lines. MTT assay was used to assess the *in vitro* anti cancer activity. The extract at various doses were treated against all the cell lines. The parameters analyzed were percentage of cytotoxicity, percentage of cell viability and IC₅₀ values were evaluated. In results the methanolic fraction of DLR showed more potent activity than the all other fractions. All the fractions does not show any cytotoxicity against the normal L6 cell lines. nHexane, chloroform fractions showed very minimal activity, the ethyl acetate fraction showed moderate activity against all the cell lines. The Methanol fraction showed significant activity against all cell lines, especially MCF 7 cell lines were more susceptible to methanol fraction. The anti cancer nature of the methanol fraction is due to the presence of phytoconstituents like flavanoid, alkaloid, triterpene etc.

Key words: *Dalbergia latifolia* Roxb, Flavonoids, Cytotoxicity, Cell viability.

INTRODUCTION

Cancer is one of the major human diseases and causes large suffering and economic loss world-wide. Chemotherapy is one of the methods of treating cancer. However the chemotherapeutic drugs are highly toxic and have devastating side effects. Various new strategies are being developed to control and treat several human cancers (Modha J and Modha N, 2007). Over 60% of anticancer drugs available in the market are of natural origin. Natural products are also the lead molecules for many of the drugs that are in use (Cragg GM *et al.*, 1997). Therefore, the phytochemicals present in several herbal products and plants may have the potential to act as preventive or therapeutic agents against various human cancer. The increased

popularity of herbal remedies for cancer therapy perhaps can be attributed to the belief that herbal drugs provide benefit over that of allopathy medicines while being less toxic (Gupta S *et al.*, 2004). Since the conventional therapies have devastating side effects, there is a continuous need for search of new herbal cures of cancer (Aquil F *et al.*, 2006)

Nature is an important source of medicinal products. Subsequently, numerous useful drugs have been developed from natural sources. In particular, plants provide valuable anticancer agents with novel structures and unique mechanisms of action (Cragg GM *et al.*, 2009). Evidence of such successes in natural product drug discovery includes the isolation of vinca-alkaloids, vinblastine and vincristine from the Madagascar periwinkle *Catharanthus roseus*, as well as paclitaxel from the bark of the Pacific Yew *Taxus brevifolia* (Cragg GM and Newman DJ, 2005). Various parts of the latter and other *Taxus* species are used by Native Americans for a variety of disease indications, including cancer.

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Similarly, *Dalbergia latifolia* Roxb belongs to the family of Fabaceae. It is also known as *Amerimnon latifolium*, Black Rosewood, Bombay Rosewood, East Indian Rosewood, Indian Rosewood, Indian Palisandre, Java Palisandre, Malabar Rosewood, Roseta Rosewood. In Tamil it is called as Etti. The plant is predominantly a single stemmed deciduous tree with a dome shaped crown of lush green foliage. The tree can become 20-40m tall, with a trunk diameter of 1.5-2m. The bark is gray, thin with irregular short cracks, peeling in fibrous longitudinal flakes. The leaves alternately arranged leaves are old pinnate with unequal sized leaflets originating from the same rachis. Leaflets are broadly blunt-tipped dark green above and pale below. The flowers are whitish pink in color. Its crown is oval in shape. Fruit is brown and rod like in shape. Fruit is dry and hard. Seeds contain 1-4 smooth brown seeds and do not open at maturity. (Anonymus, 1832; Matthew, 1983; Bhat KM, 1994).

MATERIALS AND METHOD:

Plant collection and identification

The plant *Dalbergia latifolia* Roxb. was collected from kolli hills, Namakkal district of Tamilnadu. These plants grow abundantly at high altitudes and in shady regions of dense forests which needs recurrent rainfall. The plant was then authenticated by the Botanist, The Rabinet herbarium centre, St. Joseph College, Tiruchirapalli and BSI Coimbatore.

Preparation of the extract

The plant materials were dried in shade coarsely powdered and passed through sieve No.40 and was used for the extraction separately. The shade dried coarsely powdered plants were extracted with 80% aqueous ethanol by maceration at room temperature for 72 hours. After maceration, the extract was filtered, concentrated to dryness in rotavapour under reduced pressure and controlled temperature (40-50⁰C). Dark yellowish brown colour residues were obtained and the extract was fractionated with the various solvents like nHexane, chloroform, ethyl acetate and methanol based on their polarity. The residues were then stored in separate desiccators and used for this experiment (Bently and Driver's, 1965; Harbourne JB, 1984; Hebert E Brain and Ellery W Kenneth, 1984).

IN VITRO CYTOTOXIC ACTIVITY:

Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically²³⁻²⁴.

Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Cell proliferation kit

MTT (Roche applied sciences, Cat. No. 11465 007 001)

Media

DMEM (Dulbecoo's Modified Eagels medium, high glucose), DMEM (Dulbecco's Modified Eagels medium, low glucose), FBS (Fetal Bovine Serum) (Bioclot, Lot No: 07310).

Glasswares and plastic wares

96-well micro titer plate, Tissue culture flasks, Falcon tubes, Reagent bottles

Equipments

Fluorescence inverted microscope (Leica DM IL), Biosafety cabinet classII (Esco), cytotoxic safety cabinet (Esco), CO2 incubator (RS Biotech, mini galaxy A), Sciences; Veer Narmad South Gujarat University, Surat by Dr. Minoobhai Parabia, Dr. Ritesh Vaidh.

Cell line used for cytotoxicity screening

Rat skeletal muscle cell line (L6), Cervical cancer cell line (HeLa), Breast cancer cell line (MCF 7), Hepato cellular carcinoma cell line (HEP G2), *Ehrlich's Ascites Carcinoma* cell line (EAC), all the cell lines brought from amala cancer institute Thrissur, Deep freezer, ELISA plate reader (Thermo), Micropipettes (Eppendorff), RO water system (Millipore).

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakcells/ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37°C in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37oC in 5% CO2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm (Plumb JA, 2004). The percentage growth inhibition was calculated using the formula below:

The percentage growth inhibition was calculated using following formula,

$$\% \text{ Cell Inhibition} = 100 - \{(At-Ab)/(Ac- Ab)\} \times 100$$

Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control

Data interpretation

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

$$\% \text{ Cell Survival} = \{(At-Ab)/(Ac-Ab)\} \times 100$$

$$\% \text{ Cell Inhibition} = 100 - \text{Cell Survival}$$

RESULT AND DISCUSSION

All the fractions were tested against various cell lines. All the fractions did not show any cytotoxicity against the normal L6 cell line. The fractions showed the IC₅₀ values of 2000 µg/ml. the results were shown in the tables (1,6,11,16), Figure (1,2,11,12,21,22,31,32). The n-Hexane fraction showed minimal activity against MCF-7, HEP-G, HELA cell lines. (Table 3-5) (Figure 5-10) and showed moderate activity against EAC cell line. (Table 2) (Figure 3, 4). The chloroform fraction showed moderate activity against all the four cancer cell lines (Table 7-10) (Figure 13-20). The ethyl acetate fraction showed significant cytotoxic activity against EAC and MCF-7 cell lines (Table 12,13) (Figure 23-26), moderate activity against the HEP-G and HELA cell lines. (Table 14,15) (Figure 27-30). The methanolic fraction showed significant activity against all the cell lines, especially the MCF-7 cell lines were more susceptible to the MFDLR. (Table 18) (Figure 35, 36) All the other cell lines also showed high degree of cytotoxicity when compared to the all other fractions (Table 17, 19,20) (Figure 33,34, 37-40).

Table 1. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. on L6 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.17±0.21	0.90±0.12	2000
2.	15	89.52±0.23	10.53±0.21	
3.	30	80.36±0.17	19.72±0.24	
4.	60	71.60±0.32	28.4±0.52	
5.	120	69.22±0.25	30.86±0.51	
6.	240	65.43±0.65	34.66±0.24	
7.	500	58.35±0.31	41.73±0.54	
8.	1000	55.63±0.24	44.43±0.51	
9.	1500	54.23±0.51	45.83±0.32	
10.	2000	43.65±0.32	56.43±0.15	

Table 2. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. on EAC cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.14±0.01	0.86±0.10	1250
2.	15	96.54±0.21	3.46±0.20	
3.	30	87.30±0.23	12.70±0.31	
4.	60	82.61±0.33	17.39±0.21	
5.	120	75.22±0.36	24.78±0.03	
6.	240	69.41±0.31	30.59±0.41	
7.	500	60.30±0.32	39.70±0.10	
8.	1000	48.60±0.24	51.40±0.26	
9.	1500	40.29±0.28	59.71±0.25	
10.	2000	31.66±0.29	68.34±0.26	

Table 3. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. On MCF 7 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	98.50±0.21	1.50±0.22	1000
2.	15	91.51±0.23	8.49±0.12	
3.	30	85.30±0.22	14.70±0.24	
4.	60	79.61±0.12	20.39±0.16	
5.	120	72.20±0.14	27.80±0.22	
6.	240	60.45±0.32	39.55±0.14	
7.	500	55.30±0.28	44.70±0.27	
8.	1000	42.62±0.31	57.69±0.17	
9.	1500	36.22±0.22	63.78±0.27	
10.	2000	31.60±0.24	68.40±0.32	

Table 4. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HEP G2 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	98.15±0.11	1.85±0.10	2000
2.	15	92.50±0.15	7.50±0.17	
3.	30	81.35±0.25	18.65±0.21	
4.	60	75.66±0.23	24.36±0.17	
5.	120	68.26±0.01	31.74±0.34	
6.	240	60.45±0.19	39.64±0.25	
7.	500	58.30±0.24	41.70±0.22	
8.	1000	56.62±0.21	43.45±0.14	
9.	1500	54.22±0.51	45.85±0.45	
10.	2000	45.62±0.32	54.40±0.31	

Table 5. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HeLa cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.17±0.21	0.90±0.12	2000
2.	15	89.52±0.23	10.53±0.21	
3.	30	80.36±0.17	19.72±0.24	
4.	60	71.60±0.32	28.4±0.52	
5.	120	69.22±0.25	30.86±0.51	
6.	240	65.43±0.65	34.66±0.24	
7.	500	58.35±0.31	41.73±0.54	
8.	1000	55.63±0.24	44.43±0.51	
9.	1500	54.23±0.51	45.83±0.32	
10.	2000	43.65±0.32	56.43±0.15	

Table 6. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	98.15±0.11	1.85±0.10	2000
2.	15	92.50±0.15	7.50±0.17	
3.	30	81.35±0.25	18.65±0.21	
4.	60	75.66±0.23	24.36±0.17	
5.	120	68.26±0.01	31.74±0.34	
6.	240	60.45±0.19	39.64±0.25	
7.	500	58.30±0.24	41.70±0.22	
8.	1000	56.62±0.21	43.45±0.14	
9.	1500	54.22±0.51	45.85±0.45	
10.	2000	45.62±0.32	54.40±0.31	

Table 7. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	97.19±0.01	2.90±0.01	1000
2.	15	90.53±0.12	9.52±0.65	
3.	30	85.30±0.32	14.72±0.92	
4.	60	79.62±0.23	20.40±0.98	
5.	120	70.23±0.12	29.80±0.25	
6.	240	65.43±0.21	34.62±0.64	
7.	500	60.31±0.12	39.70±0.65	
8.	1000	42.62±0.32	57.42±0.65	
9.	1500	30.21±0.12	69.81±0.36	
10.	2000	22.62±0.36	77.41±0.32	

Table 8. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On MCF 7 cell lines using MTT assay:

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.10±0.32	0.90±0.01	1000
2.	15	90.55±0.65	9.52±0.32	
3.	30	84.33±0.65	15.73±0.21	
4.	60	76.66±0.24	23.40±0.25	
5.	120	69.25±0.36	20.82±0.59	
6.	240	60.19±0.66	39.93±0.79	
7.	500	54.39±0.58	45.71±0.81	

8.	1000	45.63±0.81	54.43±0.95
9.	1500	32.29±0.79	67.83±0.56
10.	2000	21.63±0.77	78.40±0.21

Table 9. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HEP 2G cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.40±0.65	0.60±0.58	1250
2.	15	90.59±0.72	9.56±0.45	
3.	30	80.33±0.29	19.76±0.59	
4.	60	73.66±0.56	26.40±0.86	
5.	120	64.26±0.65	35.83±0.55	
6.	240	59.46±0.22	40.63±0.29	
7.	500	52.33±0.28	47.73±0.15	
8.	1000	48.69±0.91	51.43±0.45	
9.	1500	40.20±0.38	59.89±0.67	
10.	2000	20.63±0.81	79.46±0.59	

Table 10. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HeLa cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.50±0.55	0.50±0.01	1250
2.	15	89.16±0.25	10.90±0.31	
3.	30	80.80±0.69	19.24±0.64	
4.	60	72.66±0.54	27.44±0.53	
5.	120	65.59±0.39	34.57±0.57	
6.	240	60.46±0.82	39.63±0.32	
7.	500	54.34±0.91	45.79±0.52	
8.	1000	48.69±0.29	52.46±0.45	
9.	1500	40.53±0.42	59.58±0.21	
10.	2000	22.66±0.51	77.46±0.45	

Table 11. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.17±0.21	0.90±0.12	2000
2.	15	89.52±0.23	10.53±0.21	
3.	30	80.36±0.17	19.72±0.24	
4.	60	71.60±0.32	28.4±0.52	
5.	120	69.22±0.25	30.86±0.51	
6.	240	65.43±0.65	34.66±0.24	
7.	500	58.35±0.31	41.73±0.54	
8.	1000	55.63±0.24	44.43±0.51	
9.	1500	54.23±0.51	45.83±0.32	
10.	2000	43.65±0.32	56.43±0.15	

Table 12. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On EAC cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.10±0.10	0.93±0.23	750
2.	15	92.51±0.21	7.50±0.21	
3.	30	84.33±0.23	15.76±0.37	
4.	60	75.64±0.36	24.46±0.81	
5.	120	70.65±0.32	29.49±0.71	
6.	240	63.45±0.65	36.65±0.34	
7.	500	49.56±0.25	50.52±0.35	
8.	1000	40.64±0.35	59.45±0.45	
9.	1500	32.20±0.30	67.86±0.32	
10.	2000	20.65±0.33	79.40±0.21	

Table 13. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on MCF 7 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.80±0.10	0.23±0.21	750
2.	15	96.55±0.20	3.56±0.28	
3.	30	82.34±0.32	17.76±0.27	

4.	60	75.65±0.33	24.45±0.31	
5.	120	68.20±0.30	21.83±0.37	
6.	240	60.54±0.24	39.54±0.44	
7.	500	48.36±0.28	51.76±0.29	
8.	1000	40.65±0.39	59.43±0.24	
9.	1500	30.20±0.37	69.85±0.25	
10.	2000	19.63±0.55	80.41±0.30	

Table 14. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HEP G2 cell lines using MTT assay:

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.30±0.10	0.70±0.11	1000
2.	15	92.53±0.32	7.53±0.50	
3.	30	84.35±0.24	15.75±0.45	
4.	60	76.66±0.54	23.40±0.46	
5.	120	66.20±0.34	35.83±0.35	
6.	240	58.46±0.33	41.63±0.27	
7.	500	52.34±0.28	47.73±0.29	
8.	1000	44.64±0.24	55.43±0.24	
9.	1500	33.26±0.14	66.83±0.54	
10.	2000	18.63±0.28	81.40±0.12	

Table 15. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HeLa cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.49±0.11	0.66±0.10	1000
2.	15	89.90±0.21	10.15±0.28	
3.	30	80.53±0.22	19.50±0.33	
4.	60	74.60±0.39	25.43±0.39	
5.	120	66.55±0.28	33.56±0.54	
6.	240	60.26±0.34	39.86±0.34	
7.	500	53.34±0.54	46.76±0.42	
8.	1000	48.96±0.26	52.12±0.52	
9.	1500	31.87±0.66	68.21±0.32	
10.	2000	20.60±0.23	79.40±0.20	

Table 16. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	97.14±0.01	2.93±0.10	2000
2.	15	93.55±0.32	6.56±0.35	
3.	30	84.35±0.32	15.73±0.25	
4.	60	77.66±0.01	22.46±0.15	
5.	120	66.26±0.28	33.83±0.31	
6.	240	61.46±0.74	38.63±0.60	
7.	500	58.36±0.32	41.75±0.25	
8.	1000	55.66±0.23	44.46±0.20	
9.	1500	51.20±0.20	48.86±0.10	
10.	2000	42.63±0.30	57.42±0.32	

Table 17. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.10±0.10	0.93±0.23	45
2.	15	65.51±0.21	34.50±0.21	
3.	30	49.33±0.23	50.76±0.37	
4.	60	40.64±0.36	59.46±0.81	
5.	120	32.65±0.32	67.49±0.71	
6.	240	20.45±0.65	79.65±0.34	
7.	500	11.56±0.25	88.52±0.35	
8.	1000	1.64±0.35	98.65±0.45	
9.	1500	0.90±0.30	99.10±0.32	
10.	2000	0.05±0.33	99.05±0.21	

Table 18. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on MCF 7 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.11±0.10	0.89±0.23	30
2.	15	63.51±0.21	36.50±0.21	
3.	30	45.33±0.23	54.76±0.37	
4.	60	38.64±0.36	61.46±0.81	
5.	120	30.65±0.32	69.49±0.71	
6.	240	21.45±0.65	78.65±0.34	
7.	500	14.56±0.25	85.52±0.35	
8.	1000	1.61±0.35	98.39±0.45	
9.	1500	0.90±0.30	99.10±0.32	
10.	2000	0.03±0.33	99.07±0.21	

Table 19. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HEP G2 cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	97.29±0.0	2.71±0.05	120
2.	15	80.44±0.36	19.56±0.21	
3.	30	75.75±0.29	24.25±0.43	
4.	60	64.64±0.32	35.46±0.42	
5.	120	42.45±0.26	57.63±0.21	
6.	240	31.46±0.54	68.64±0.26	
7.	500	22.43±0.48	77.63±0.44	
8.	1000	11.76±0.49	88.34±0.24	
9.	1500	3.20±0.31	96.85±0.66	
10.	2000	0.40±0.20	99.60±0.25	

Table 20. Evaluation of cytotoxicity and cell viability for Methanol fraction of hydro alcoholic extract of *Dalbergia latifolia* roxb. On HeLa cell lines using MTT assay

S.no	Conc (µg/ml)	% Cell Viability	% Cytotoxicity	IC ₅₀ Value (µg/ml)
1.	Control	99.29±0.04	0.71±0.06	120
2.	15	80.66±0.35	19.43±0.20	
3.	30	76.75±0.22	23.25±0.44	
4.	60	66.64±0.32	33.46±0.41	
5.	120	45.45±0.29	54.63±0.21	
6.	240	33.46±0.55	66.64±0.27	
7.	500	25.43±0.47	74.63±0.45	
8.	1000	18.76±0.43	81.34±0.25	
9.	1500	7.20±0.30	92.85±0.66	
10.	2000	0.60±0.23	99.40±0.29	

Figure 1. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. on L6 cell lines

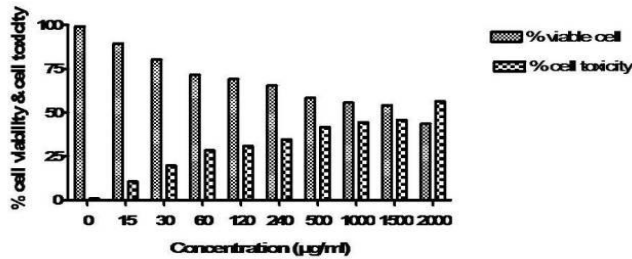


Figure 2. Effect of n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. on L6 cell lines

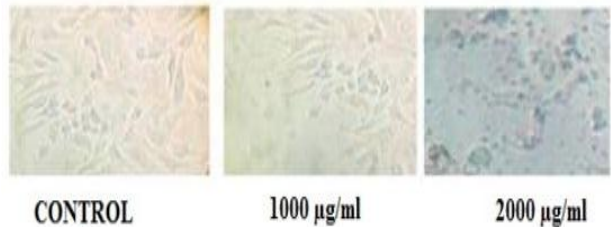


Figure 3. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. on EAC cell lines

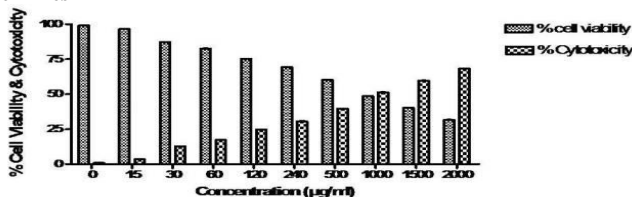


Figure 4. Effect of n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. on EAC cell lines

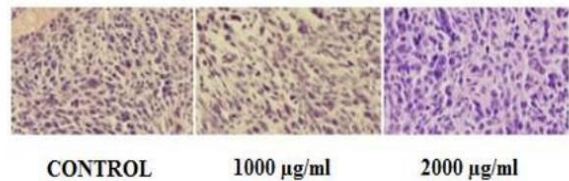


Figure 5. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. On MCF 7 cell lines.

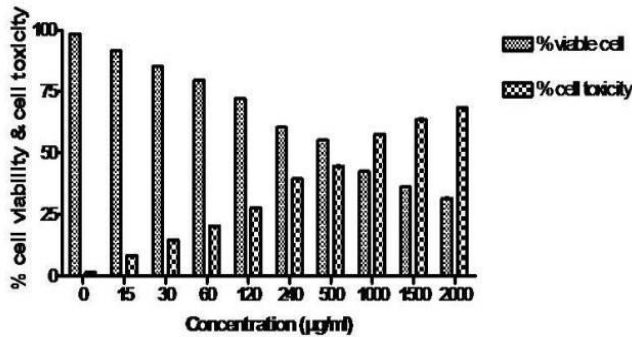


Figure 6. Effect of n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* Roxb. On MCF 7 cell lines.

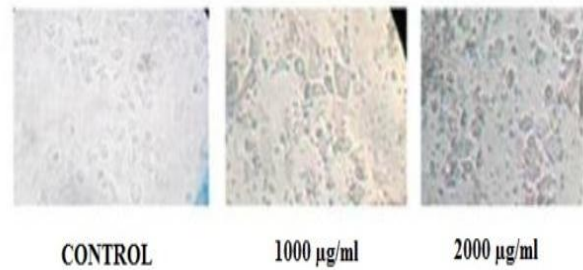


Figure 7. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HEP G2 cell lines.

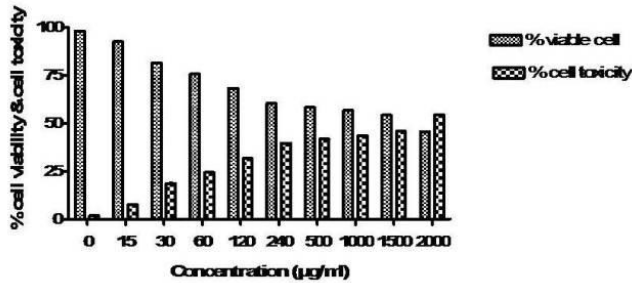


Figure 8. Effect of n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HEP G2 cell lines.

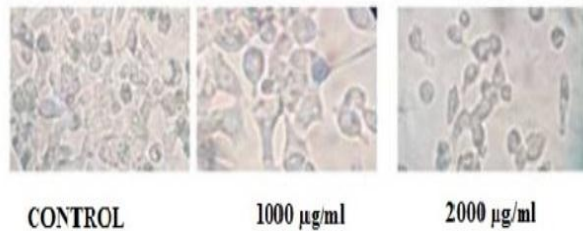


Figure 9. Evaluation of Cytotoxicity and Cell Viability for n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HeLa cell lines.

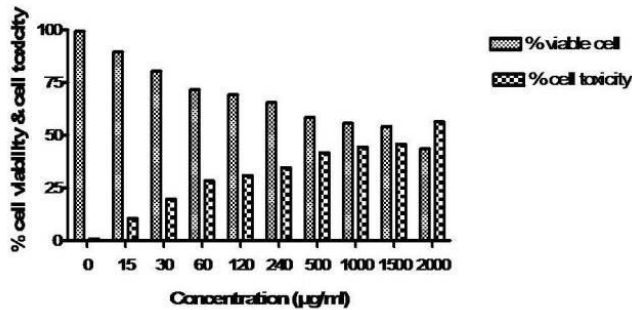


Figure 10. Effect of n-Hexane fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HeLa cell lines.

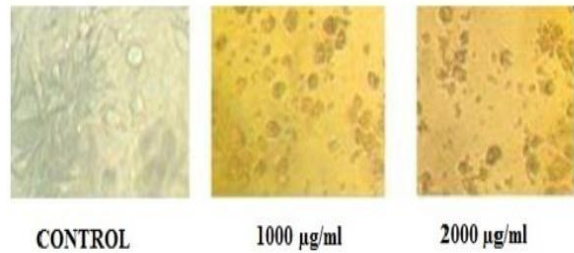


Figure 11. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines.

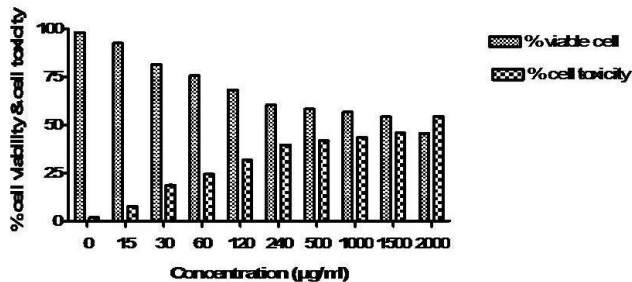


Figure 12. Effect of Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines.

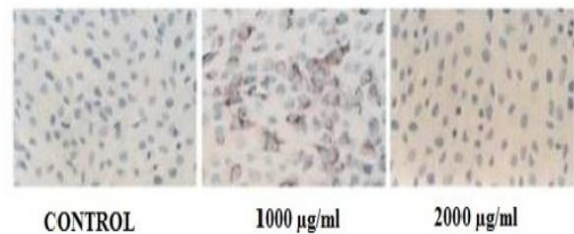


Figure 13. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines.

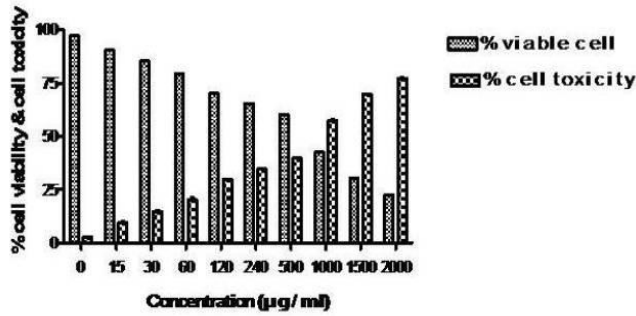


Figure 14. Effect of Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines.

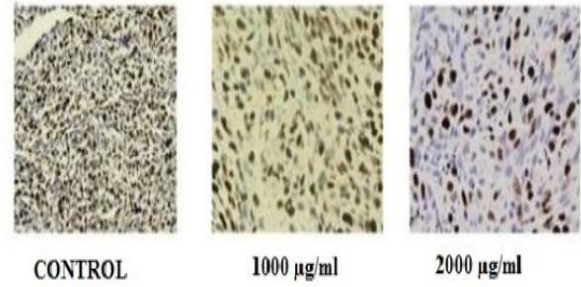


Figure 15. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On MCF 7 cell lines

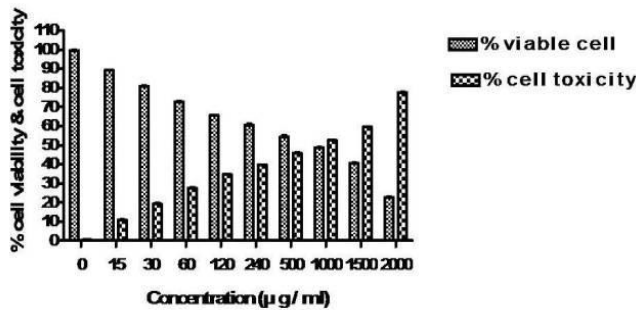


Figure 16. Effect of Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On MCF 7 cell lines

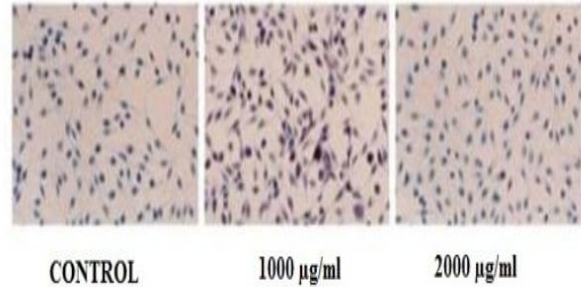


Figure 17. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HEP G2 cell lines.

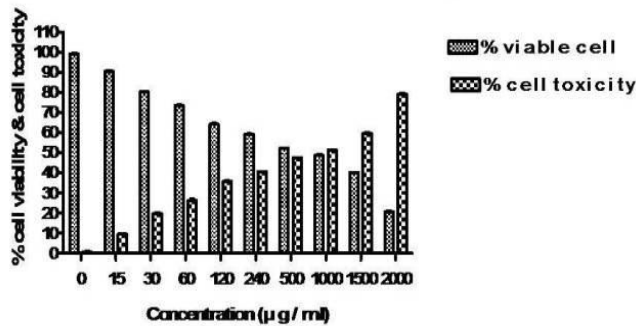


Figure 18. Effect of Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HEP- G2 cell lines.

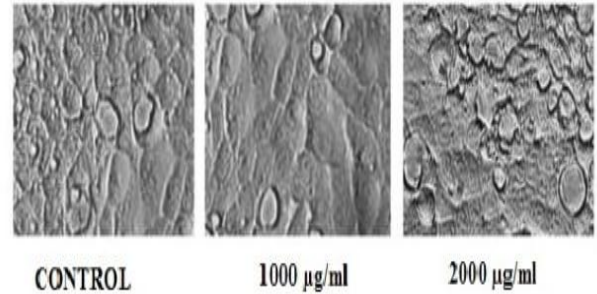


Figure 19. Evaluation of Cytotoxicity and Cell Viability for Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HeLa cell lines.

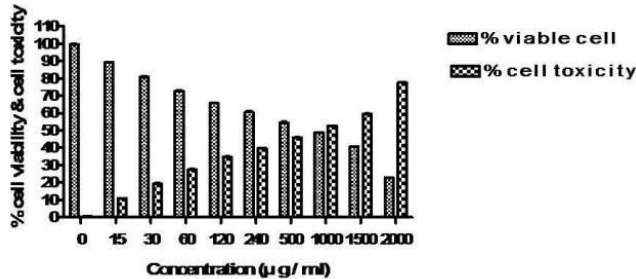


Figure 20. Effect of Chloroform fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HeLa cell lines.

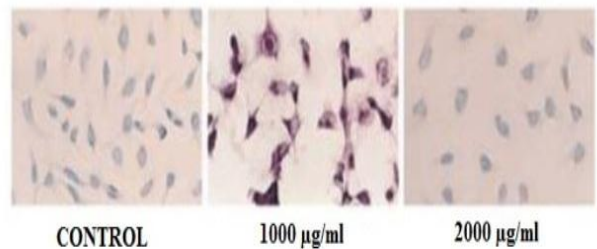


Figure 21. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines.

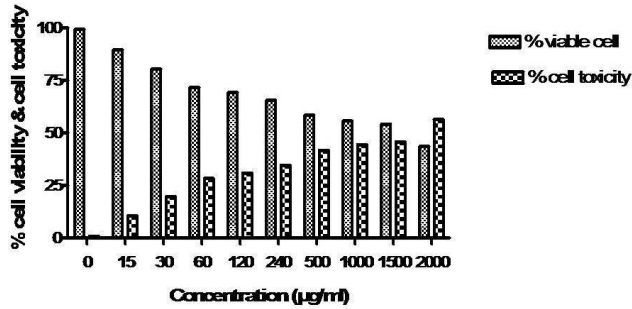


Figure 22. Effect of Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines

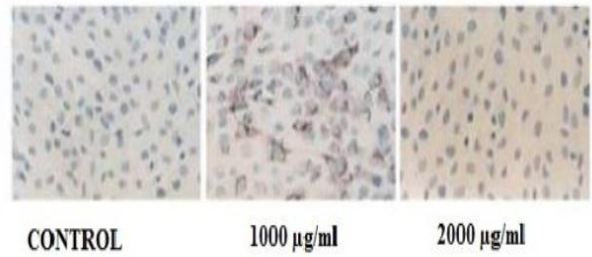


Figure 23. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines.

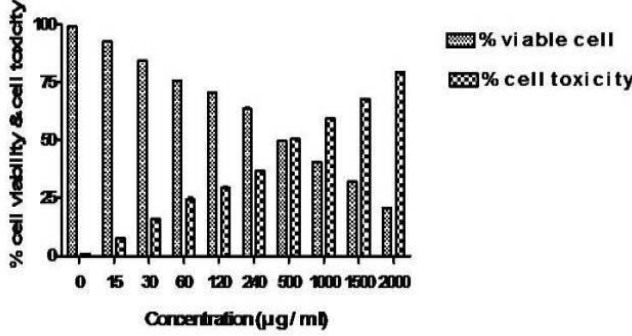


Figure 24. Effect of Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines.

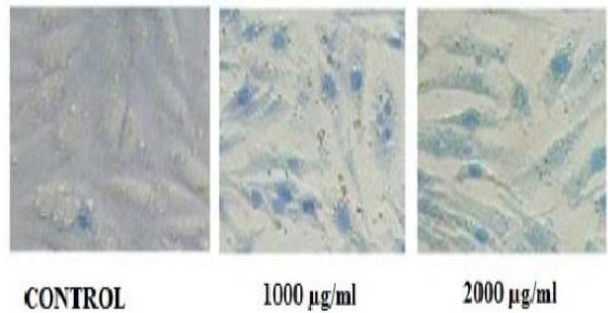


Figure 25. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on MCF 7 cell lines

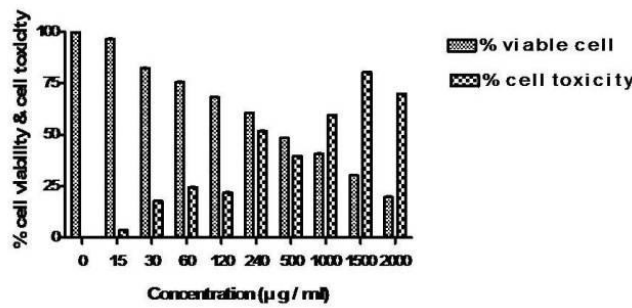


Figure 26. Effect of Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on MCF 7 cell lines.

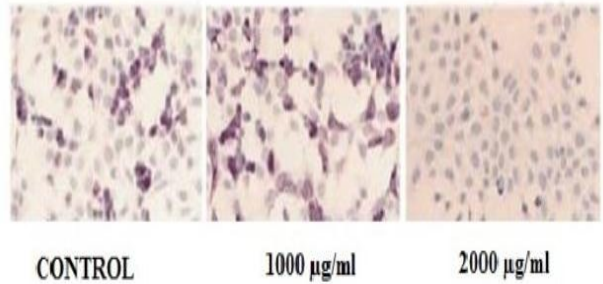


Figure 27. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HEP G2 cell lines

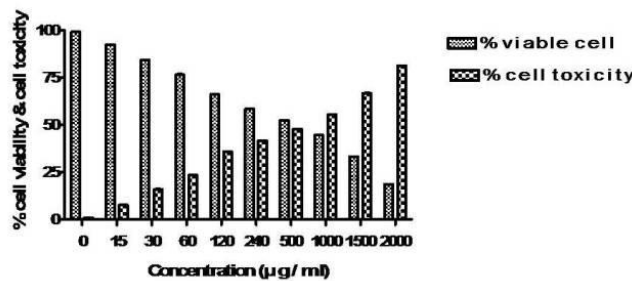


Figure 28. Effect of Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HEP G2 cell lines.

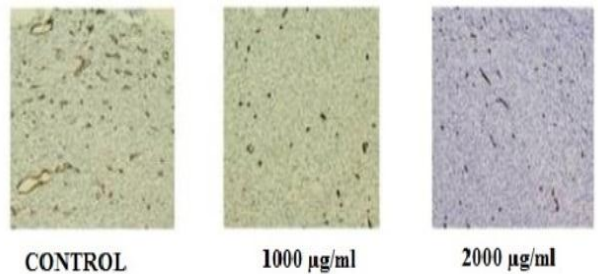


Figure 29. Evaluation of Cytotoxicity and Cell Viability for Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HeLa cell lines.

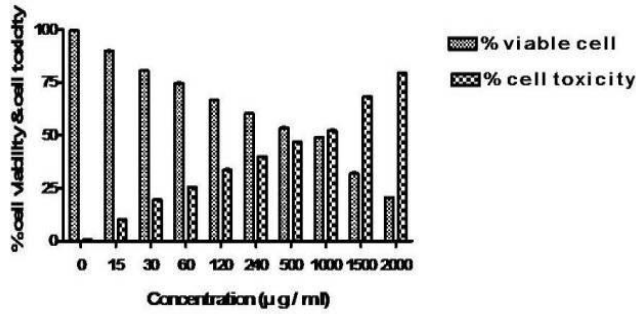


Figure 30. Effect of Ethyl Acetate fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on HeLa cell lines.

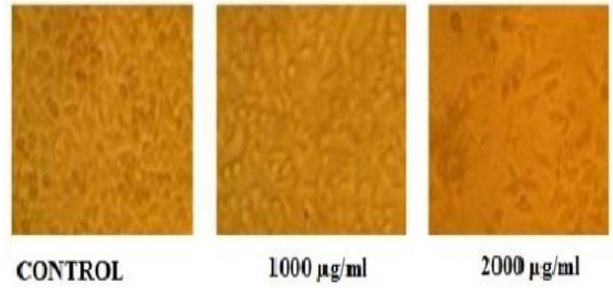


Figure 31. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines.

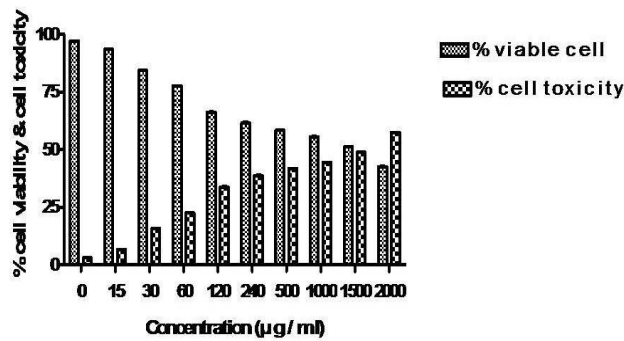


Figure 32. Effect of Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on L6 cell lines.

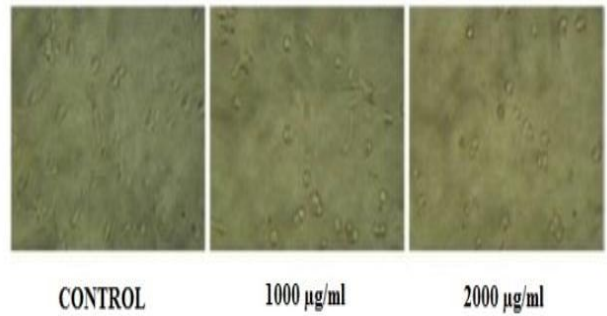


Figure 33. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines.

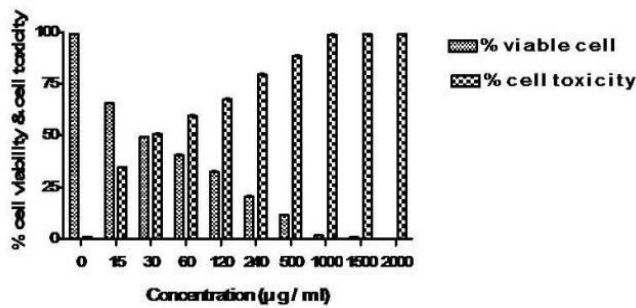


Figure 34. Effect of Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on EAC cell lines.

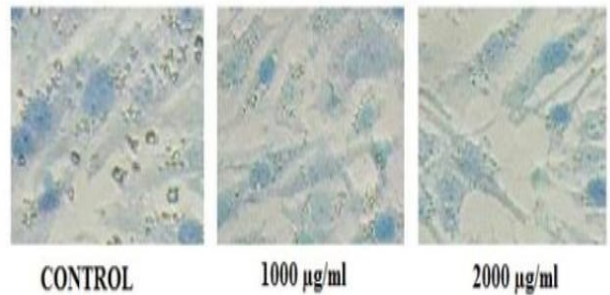


Figure 35. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on MCF 7 cell lines.

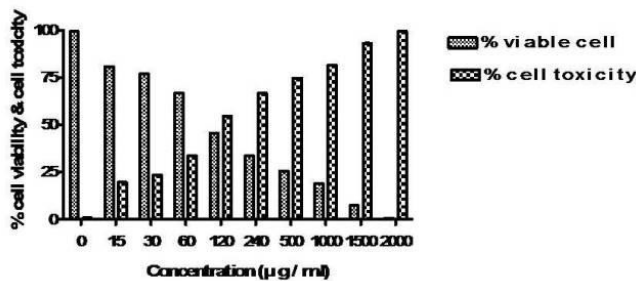


Figure 36. Effect of Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. on MCF 7 cell lines.

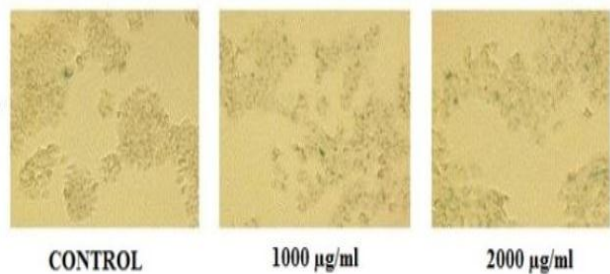


Figure 37. Evaluation of Cytotoxicity and Cell Viability for Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HEP G2 cell lines.

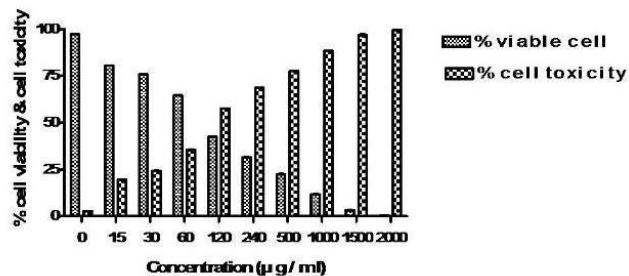


Figure 38. Effect of Methanol fraction of Hydro Alcoholic extract of *Dalbergia latifolia* roxb. On HEP G2 cell lines.

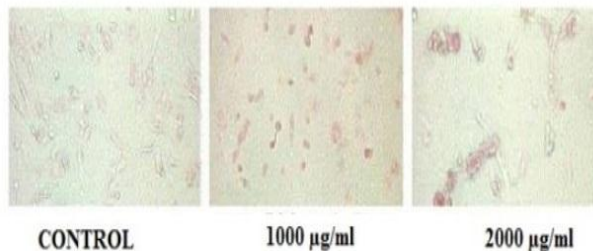


Figure 39. Evaluation of cytotoxicity and cell viability for Methanol fraction of hydro alcoholic extract of *Dalbergia latifolia* roxb. On HeLa cell lines.

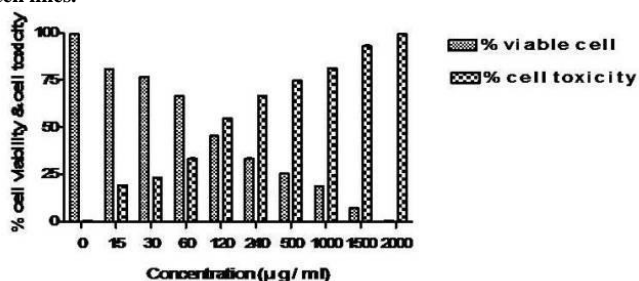
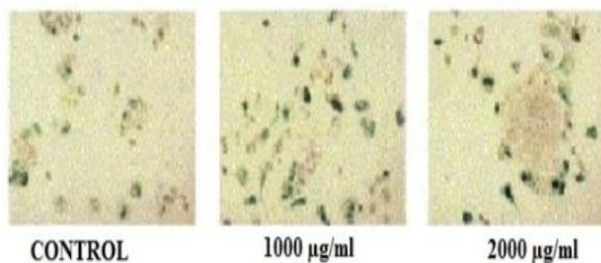


Figure 40. Effect of Methanol fraction of hydro alcoholic extract of *Dalbergia latifolia* roxb. On HeLa cell lines.



The methanolic fraction of hydro alcoholic extract of *Dalbergia latifolia* Roxb has more significant anti cancer activity when compared to the all other fractions of hydro alcoholic extract of *Dalbergia latifolia* Roxb. it is mainly because of the phytoconstituents present in the fraction. The phytochemical study revealed

the presence of various phytoconstituents like flavanoids, alkaloids, triterpenoids, tannins etc. the further study is planned to isolate the active constituent responsible for the anti cancer nature of the methanolic fraction or hydro alcoholic extract of *Dalbergia latifolia* Roxb.

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