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In Vitro Cytotoxicity Analysis of *Blumea oxyodonta* DC. Leaves

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Abstract

Plants are the best source for making medicines. Plants can be used to cure diseases in a variety of ways depending on their medicinal qualities, pharmacological actions, and active bio chemicals present in them. In this work, *Blumea oxyodonta* DC. were evaluated for *in vitro* cytotoxicity test using ethanolic crude extract of the leaves of *B. oxyodonta* with DLA cells and normal spleen cells. The invitro cytotoxicity analysis using ethanolic crude extract showed cytotoxic effect of *B. oxyodonta*. Result showed that cytotoxic activity towards normal cell with IC50 value 1512.71875 and cytotoxicity towards DLA cells which have IC50 value 263.824675. Drug concentration has been given as 12.5, 25, 50, 100, 150, and 200 mg/mL. The percentage of cell death is increasing with an increase in drug concentration in in vitro cytotoxicity analysis on DLA cells. This confirms the cytotoxic activity of ethanolic extracts of *Blumea oxyodonta* DC. leaves.

***Key words*:** *B. oxyodonta, In vitro,* Cytotoxicity, Ethanolic crude extract, Medicines

For the welfare of humans, plants are essential. For basic requirements like food, medicine, lumber, etc., man depends on plants. Man has been classifying and using specific plants as edible, dangerous, therapeutic, etc. from the beginning of time. The plant kingdom is a veritable gold mine of potential medicines, and awareness of the significance of medicinal plants has grown recently. Plant-based medications, are widely accessible, less priced, effective, safe, and rarely cause negative effects [1]. Looking at the plants that have been used for medical purposes for thousands of years is the most apparent choice for investigating the present search for therapeutically effective and new drugs, such as anticancer treatments.

Numerous studies have shown the effectiveness of using medicinal herbs to treat cancer. Consequently, there is an increasing demand for the development and study of naturally derived compounds that can be used as anticancer treatments, with a focus on those derived from plant species and their natural products. Cancer is one of the most lethal diseases. Cancer is a group of illnesses characterized by unchecked cell development that invades and disrupts the tissues around [2]. As supplementary therapy for cancer, the utilization of medicinal plants and the phytocompounds generated from them is becoming more popular. Medications made from plants are frequently utilized to treat cancer since they are affordable and secure [3]. Since they are created from plants, they are often more readily tolerated and do not harm healthy human cells. The selection of plant extracts with potential anti-cancer effects for future research can be based on significant preliminary findings from cytotoxicity screening models. DNA alterations, which direct how the cells grow and divide, are the first step in the development of cancer. The majority of DNA mutations can be repaired by normal cells, but those that cannot are what cause malignant cells to develop and grow.

Whole plant extract *Blumea oxyodonta* DC. exhibit antioxidant and anti-inflammatory activities, cytotoxicity assay of ethanolic extract through MTT assay in HeLa cells reveals its strong cytotoxic activity [4]. Also, the phytochemical screening shows the presence of tannins, flavonoids, saponins and phenolics. The terpenoid profiling of *Blumea oxyodonta* DC has also done [5]. Sixty-one compounds were characterized and identified according to their mass spectra and their relative retention. It was found out the presence of antioxidant and anticancer activity of methanolic and ethanolic extracts of *Blumea lacera* [6]. When compared to ethanolic extract, the methanolic extract of the leaves demonstrated effective antioxidant activity. Additionally, methanolic extract had stronger anticancer efficacy than ethanolic extracts. It was also reported that *Blumea balsamifera* Lin. (DC) leaf extract have antibacterial and anticancer activities [7]. HES extract and HE extract shows difference in anticancer potential in MTT assay. Strong cytotoxicity is shown by ethyl acetate (HES) extract against HeLa cell lines, and moderate activity is shown by MCF-7 cell lines. Meanwhile, HeLa and MCF-7 cells were moderately cytotoxic to HE extracts.

It was revealed that n-hexane extract of *Blumea balsamifera* to be a moderately cytotoxic agent with an IC_{50} of 44.47 ppm and a weak antioxidant with an IC_{50} of 281.707 ppm [8]. Two phytoconstituents that are known to have anticancer properties, borneol L (33.77%) and veridiflorol (22.57%), were found to dominate the GC chromatogram. N-hexane extract

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from *Blumea balsamifera* leaves has been shown to have anticancer potential by phytochemical screens. Studies shows that antioxidant and cytotoxic activity of *Blumea lanceolaria*, antioxidant activity with IC_{50} value 55.56 mg/mL and 70.60 mg/mL with respect to DPPH radical scavenging and nitric oxide scavenging [9]. The cytotoxic activity through MTT assay on HeLa cell line suggest leaf extract of *Blumea lanceolaria* exhibit moderate cytotoxicity. The aerial parts of a *Blumea lacera* showed mild analgesic, anti-inflammatory and Antidiarrheal properties [10]. It could be used as a diuretic, antibacterial, anthelmintic and cytotoxic drug to justify the use of the plant in traditional medicine.

In a straightforward and *ecologically* friendly method, the anti-cancerous capabilities of silver nanoparticles (AgNPs) synthesized using *Blumea lacera* leaf extract was also examined [11]. They assert that AgNPs generated by *Blumea lacera* have excellent potential for use as safe anti-cancer medications. Silver and iron nanoparticles were used to conduct biological screening on *Blumea eriantha* DC. leaves, and the results demonstrated strong antioxidant, antibacterial, and cytotoxic activities [12].

MATERIALS AND METHODS

Fresh *Blumea oxyodonta* DC. leaves were taken in January 2023 from the Carmel College campus in Mala, Thrissur. The leaves were cleaned by washing them in water and then let to air dry in the shade. Using a mixer, the dried leaves were reduced to a fine powder and kept in an airtight container. Preparation of crude extract for in vitro cytotoxic testing. The plant parts were air dried at ambient temperature for about 20 days before being ground into powder. Dry powder was extracted from ethanol at room temperature. Dry ethanolic extracts were created after the solvent was evaporated. The appropriate extract solutions were then made by dissolving dry ethanolic extracts in dimethyl sulfoxide (DMSO).

Invitro cytotoxicity analysis

- The test compound was studied for short term in vitro cytotoxicity using Dalton's Lymphoma Ascites cells (DLA).
- The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal cell line.
- Cell viability was determined by trypan blue exclusion method.
- Viable cells suspension (1X106 cells in 0.1m1) was added to tubes containing various concentrations of the test compounds and the volume was made up to l ml using phosphate buffered cell line (PBS).
- Control tube contained only cell suspension.
- These assay mixtures were incubated for 3 hours at 37 °C.
- Further cell suspension was mixed with 0.lml of trypan blue and kept for 2-3 minutes and loaded on a haemocytometer.
- Dead cells take up the blue colour of trypan blue while live cells don not take up the dye.
- The number of stained and unstained cells were counted separately:

$$\% \text{ of cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of dead cells + No. of live cells}} \times 100$$

RESULTS AND DISCUSSION

Invitro cytotoxicity analysis

Invitro cytotoxicity of ethanolic extract of *Blumea oxyodonta* DC. was carried out by trypan blue exclusion method. Cytotoxicity towards both DLA cells and Normal cells was analyzed. Result showed that cytotoxic activity towards normal cell with IC50 value 1512.71875 and cytotoxicity towards DLA cells which have IC50 value 263.824675.

From the result cytotoxicity of *B. oxyodonta* DC. leaves percentage of cell death increases with increase in drug concentration when tested with Dalton Lymphoma Ascites cells. And the graph with drug concentration in X axis and% of cell death on Y axis is almost a straight line. And the maximum activity is showed when the concentration of drug was 200 µg/ml. And the minimum was showed when the concentration of drug was 12.5 µg/ml.

Test for cytotoxicity towards normal cell showed lower activity than Dalton's Lymphoma Ascites cells (DLA) cells. Cell death was same when the concentrations of drug were 12.5, 25 and 50. Then only the percentage of death increases with increasing concentration of drug.

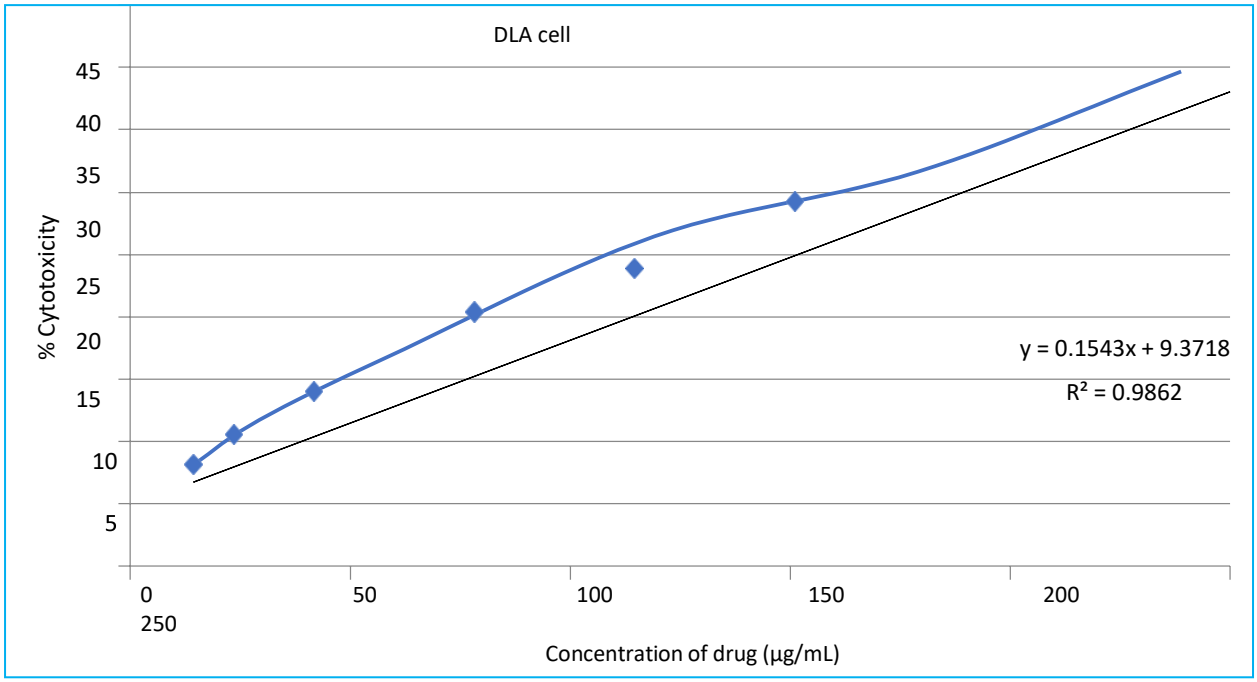
Many studies were conducted previously on cytotoxicity of different species of the genus *Blumea*. and many of them have cytotoxic activity in them. It is proved that *Blumea balsamifera* having cytotoxic activity [13]. *Blumea eriantha* DC. showed cytotoxic activity as per studies [14]. Another work found out the anticancer activity of ethanolic extract of *Blumea lacera*. Cytotoxic activity of *Blumea lanceolaria* was described in some works [15]. These are some examples for works showing cytotoxic activity of many species of *Blumea*.

Saikia *et al.* [16] also studied the cytotoxicity of whole plant of *Blumea lanceolaria*. They were tested using Hela cell line through MTT assay and result showed the IC50 value 37.93. The present study also showed the cytotoxicity of the plant and support its anticancer activity. Result of invitro cytotoxicity of ethanolic extract of *Blumea oxyodonta* DC. leaves were shown in the following (Table 1-2) and graphs in (Fig 1-2) respectively.

Table 1 Invitro cytotoxicity analysis of ethanolic extract of *B. oxyodonta* DC. using DLA cells

DLA cell line	Tube 1	Tube 2	Tube 3	Tube 4	Average	SD
Drug concentration 200 µg/mL						
Live cells	68	65	66	64	39.4	1.85
Dead cells	40	44	43	45		
Total number of cells	108	109	109	109		
Percent (%) of cytotoxicity	37	40.4	39.45	41		
Drug concentration 150 µg/mL						
Live cells	74	76	74	76	31.9	1.21
Dead cells	35	37	32	36		
Total number of cells	109	113	106	102		
Percent (%) of cytotoxicity	32	32.7	30.14	33		
Drug concentration 100µg/mL						
Live cells	78	77	76	74	27	0.69
Dead cells	30	29	27	27		

Total number of cells	108	106	103	101		
Percent (%) of cytotoxicity	28	27.4	26.21	27		
			Drug concentration 50 µg/mL			
Live cells	87	85	84	85		
Dead cells	18	19	21	17	18	1.48
Total number of cells	105	104	105	102		
Percent (%) of cytotoxicity	17	18.3	20	17		
			Drug concentration 25 µg/mL			
Live cells	94	95	94	94		
Dead cells	12	15	14	16	13.1	1.36
Total number of cells	106	110	108	110		
Percent (%) of cytotoxicity	11	13.6	12.96	15		
			Drug concentration 12.5 µg/mL			
Live cells	102	97	96	97		
Dead cells	9	11	12	10	9.69	1.28
Total number of cells	111	108	108	107		
Percent (%) of cytotoxicity	8.1	10.2	11.11	9.3		



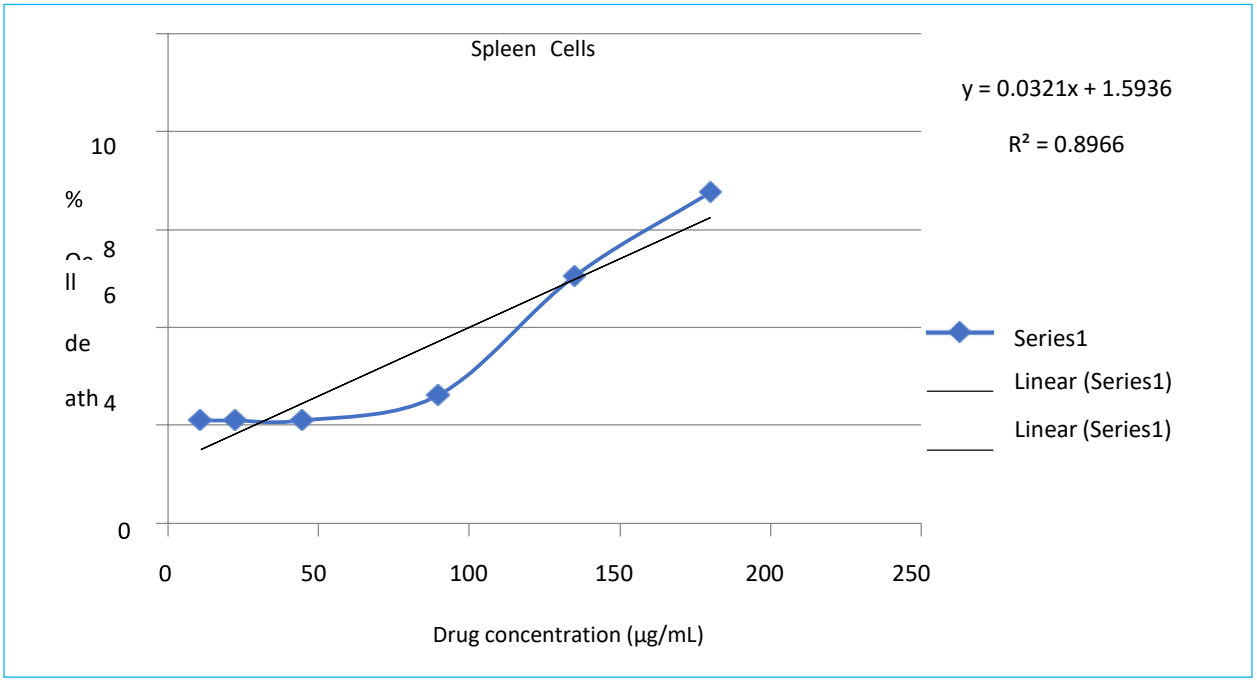
IC_{50} is calculated from the graph using the equation y=Mx + b. Y = IC_{50}
So, the equation is x = (IC_{50} – b) / M
IC_{50} of the given sample = 263.8246753

Fig 1 Graphical representation of invitro cytotoxicity of *B. oxyodonta* DC. using DLA cells

Table 2 Invitro cytotoxicity analysis of *B. oxyodonta* DC. using spleen cells

Spleen cell	Tube 1	Tube 2	Tube 3	Tube 4	Average	SD
			Drug concentration 200 µg/mL			
Live cells	102	102	103	103		
Dead cells	11	10	9	9	8.68	0.82
Total number of cells	113	112	112	112		
Percent (%) of cytotoxicity	9.7	8.93	8.0357	8		
			Drug concentration 150 µg/mL			
Live cells	104	104	104	105		
Dead cells	8	7	7	7	6.5	0.43
Total number of cells	112	111	111	112		
Percent (%) of cytotoxicity	7.1	6.31	6.31	6.3		
			Drug concentration 100 µg/mL			
Live cells	106	106	106	106		
Dead cells	5	4	3	3		0.84
Total number of cells	111	110	109	109	3.41	
Percent (%) of cytotoxicity	4.5	3.64	2.7523	2.8		

			Drug concentration 50 µg/mL			
Live cells	106	106	106	106	2.75	0
Dead cells	3	3	3	3		
Total number of cells	109	109	109	109		
Percent (%) of cytotoxicity	2.8	2.75	2.7523	2.8		
			Drug concentration 25 µg/mL			
Live cells	106	106	106	106	2.75	0
Dead cells	3	3	3	3		
Total number of cells	109	109	109	109		
Percent (%) of cytotoxicity	2.8	2.75	2.7523	2.8		
			Drug concentration 12.5 µg/mL			
Live cells	106	106	106	106	2.75	0
Dead cells	3	3	3	3		
Total number of cells	109	109	109	109		
Percent (%) of cytotoxicity	2.8	2.75	2.7523	2.8		



IC_{50} is calculated from the graph using the equation y=Mx + b. Y = IC_{50}
So, the equation is x = (IC_{50} – b) / M
IC_{50} of the given sample = 1512.71875

Fig 2 Graphical representation of invitro cytotoxicity using spleen cells

CONCLUSION

In traditional medicine, plants are the best source for treating many disorders. As a result, plants can be used to make medicines that can treat a variety of illnesses. These qualities are provided by the biochemicals found in the plant. The plants also have additional characteristics because of particular endophytic associations. Numerous secondary metabolites are produced as a result of their interaction, the majority of which are advantageous. In the present study cytotoxicity analysis on *Blumea oxyodonta* DC. leaves were carried out. Ethanolic crude extract of *B. oxyodonta* leaves was studied for short-term in vitro cytotoxicity with Dalton's Lymphoma Ascites cells (DLA) and normal Cells using the trypan blue exclusion method. Drug concentration has been given as 12.5, 25, 50, 100, 150, and 200 mg/mL. The percentage of cell death is increasing with an increase in drug concentration in in vitro cytotoxicity analysis on DLA cells. IC50 values for DLA cells are 263.8246753 and normal cells are 1512.71875. This confirms the cytotoxic activity of ethanolic extracts of *Blumea oxyodonta* DC. leaves.

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