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In Vitro Cytotoxicity Evaluation of Stem and Leaf Extract of *Ampelocissus latifolia* Planch.

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Abstract: *Ampelocissus latifolia* is a medicinal plant belonging to the family Vitaceae. It is widely used in wound healing, bone fracture, syphilis etc. Present study aimed to evaluate the *in vitro* cytotoxic effect of stem and leaf extract of *Ampelocissus latifolia* Planch. Short term cytotoxicity assay (Trypan blue method) was used to evaluate the cytotoxic effect on cancer cell lines. The results revealed the cytotoxic effects against cancer cell lines. These findings suggest the potential therapeutic applications of *A. latifolia* Planch. in the pharmaceutical and healthcare sectors.

Keywords: *Ampelocissus latifolia*, Stem, Leaf extract, *In vitro* cytotoxicity, Trypan blue, Therapeutic, Pharmaceutical

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Introduction

Herbal medicines have been used extensively throughout the world because of the low side effects and the cost-effectiveness, and have received greater attention than allopathic medicines. These herbal remedies which are made from plants are much safer alternative for treating wide range of illnesses (Ayyanar and lgnacimuthu, 2005).

As these historic healthcare systems developed well in direct relationship with nature, the traditional medicines, and specially folk herbal medicines, have become very popular worldwide. Unique chemotherapeutic drugs which are derived from plants have been discovered in large part of ethnic groups' which pave way for insights into indigenous traditional herbal treatments (Katewa and Galav, 2005).

For thousands of years of usage in traditional medicine, medicinal plants are the oldest class of medicine throughout numerous nations, world-wide. Due to the centuries-long dissemi-nation of knowledge about their advantageous qualities,

across human cultures, natural healing practices have a rich history (Khan, 2014).

A great deal of research has been carried out on the various biological traits of many plant species that are frequently used traditionally, for their bioactive components. The potential medical benefits and chemical components of these plants have been made possible by both contemporary methods like high-throughput screening and the conventional bioassay-guided natural drug discovery methodology (Marrelli, 2021). Properties and effects of natural products, originating from plants of different kinds, on human health have been studied (Sneader, 2005).

Plants that contain biological activity naturally contain chemical molecules called phytochemicals, which come from the Greek word "phyto," which means "plant." As per Hasler and Blumberg (1999), these phytochemicals give human health benefits beyond those of macronutrients and micronutrients. Plants having phytochemicals are more colorful, fragrant, and flavorful in addition to being protected against disease and other harm. In addition to working with nutrients and fibers, these substances are essential to the plant's defensive mechanism against a different illnesses and demanding circumstances (Thilagavathi *et al.*, 2015).

Main cause of treatment failures and a danger to the effectiveness of currently available medications may be the emergence and spread of drug-resistant bacteria (Mahady, 2005; Lacmata *et al*., 2012; Cheuka *et al.,* 2017). Many bacteria developed resistance so that antibiotics that were previously thought to be miraculous, no longer work against them. According to Schultz *et al*. (2020) and Fonkeng *et al*. (2015) many multidrug-resistant bacterial strains have been identified as extremely dangerous microorganisms.

Bhardwaj *et al*. (2016) opined that due to the sharp drop in approvals of novel antimicrobial medications in recent years, it is anticipated that the supply of potent antimicrobials would run out soon. For novel and effective pharmaceuticals, traditional medicine relied on medicinal plants as a source. Developments in phytochemistry and pharmaceutical chemistry have been made it easier to make plant-based active ingredients or synthetic substitutes in medicine. Dubale *et al*. (2023) opined that this is because medicinal plants have a greater variety and uniqueness of compounds than other sources.

Very rapidly and uncontrollably growing cells are the characteristic feature of cancer, which ranks third among causes of death after infectious and cardiovascular disorders. As per the studies of Supek and Lehner (2015), this disease is caused by alterations in the genetic and epigenetic pathways brought on by various microbial infections, hormonal abnormalities, and exposure to some carcinogens and mutagens. Globally, cancer is the second biggest cause of death. Cancer treatment and management have come a long way, there are still many unaddressed issues and room for improvement. Plant-based medicines and other natural cures may be able to lessen these negative effects. While certain compounds derived from plants are now used in cancer therapy, many others show promise in laboratory tests about their ability to combat cancer but still need more research (Desai *et al*., 2008). In recognition of the great potential of plants as sources for traditional medicine systematic research has been done to evaluate the efficacy of plants in treating microbial infections and cancer (Gacche *et al*., 2011).

Present study aimed to evaluate the *in vitro* cytotoxic effect of stem and leaf extract of *Ampelocissus latifolia* Planch. Short term cytotoxicity assay (Trypan blue method) was used to evaluate the cytotoxic effect on cancer cell lines.

Materials and Methods

Collection of plant materials and preparation of extract:

The aerial parts (stem and leaves) of *A. latifolia* were collected from the regions of Thrissur (India) during the vegetative stage of the plant. It is a large climbing plant with a tuberous rootstock. Its stems and branches are hollow and generally

smooth. The leaves are round or broadly heart-shaped, measuring 7-15 cm to 8-15 cm, with 3-7 lobes that are pointed and toothed. Leaf stalks are 3-5 cm long. The inflorescence forms a dense cluster on a stalk about 6-7 cm long, ending in a long forked tendril. The flowers are numerous, colored deep reddish, with five oblong petals. The fruit is spherical, black, 6-7 mm in size, typically containing two seeds, occasionally three. This wild grapevine is native to the Himalayas, ranging from Kumaun to Nepal, and also found in the Western Ghats, growing at elevations of 300-1600 m. It flowers from May to August.

Fresh and healthy parts (stem and leaf) of *A. latifolia* were selected and washed thoroughly for 3-4 times in running tap water to remove all the dust and unwanted visible particles and finally washed with sterile distilled water for proper cleaning. The samples were dried under sunlight for a few hours. The dried plant materials were powdered using an electric grinder and kept in an airtight container.

100 ml of each distilled water and ethanol was taken. 5 g *A. latifolia* powder (stem and leaf) was mixed with each of the solutions. Kept in the stirrer for proper mixing. The preparations were left overnight and then filtered. Then it was poured into beakers, labeled and the mouth of the beakers were covered to prevent evaporation.

Cytotoxicity:

Analysis Procedure:

The test compound was studied for short term *in vitro* cytotoxicity using Dalton's Lymphoma Ascites cells (DLA). The tumor cells, aspirated from the peritoneal cavity of tumor bearing mice, were washed thrice with PBS. Cell viability was determined by the trypan blue exclusion method. Viable cell suspension (1X10 cells in 0.1ml) was added to tubes containing various concentrations (12.5 -200 µg/ml) of the test compounds and the volume was made up to 1 ml using phosphate buffered cell line (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 h at 37°C. Further cell suspension was mixed with 0.1 ml of 1% trypan blue and kept for 2-3 min and loaded on a haemocytometer. Dead cells take up the blue color of trypan blue while live cells do not take up the dye. The number of stained and unstained cells were counted separately.

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

Results and Discussion

In Vitro Cytotoxicity:

At a drug concentration of 12.5 µg/ml, the percentage of cell death observed was 5.1±0.4% (Tables 1, 2; Fig. 1). As the drug concentration increased to 25 µg/ml, the percentage of cell death also increased to 6.72 ±0.8%. Similarly, at concentrations of 50 µg/ml and 100 µg/ml, the percentage of cell death further increased to 9.92±1.2% and 25.1±0.8%, respectively. The highest concentration tested, 200 µg/ml, resulted in the highest percentage of cell death at 43.7 ±2% (Tables 1, 2; Fig. 1).

Chaudhuri and Ray (2020) studied cytotoxic effect of aerial parts of *A. latifolia* and results indicated a significant and concentration-dependent decreased survivability of DL cells treated with *A. latifolia*. The present study also revealed a dose-dependent cytotoxic effect of *A. latifolia* extracts on cancer cells.

According to the study of Pettit (2008), *Ampelocissus sp*. roots for cancer cell growth inhibitory components led to the isolation of a new acetogenin characterized as 22-epicala-mistrin (1) employing primarily 2D NMR and high-resolution mass spectral analysis. Two other antineoplastic constituents proved to be the known acetogenin uvaribonin (2) and chalcone (3). Constituents 1–3 were all found to show significant cancer cell growth inhibitory activity against a panel of human cancer cell lines. The present study also proved the cytotoxic activity of *A. latifolia* stem and leaf extract.

Table 1: *In vitro* cytotoxicity of *A. latifolia* using DLA cell lines

DLA Cell Line						
Drug concentration (µg/ml)						
	200 µg /ml				**AVG**	**SD**
Live cells	56	60	59	62		
Dead cells	48	45	47	44		
Total no. of cells	104	105	106	106		
% cytotoxicity	46.2	43	44	42	43.7	2
	100 µg /ml					
Live cells	78	79	78	78		
Dead cells	26	25	27	27		
Total no. of cells	104	104	105	105		
% cytotoxicity	25	24	26	26	25.1	0.8
	50 µg /ml					
Live cells	94	95	95	97		
Dead cells	10	11	12	9		
Total no. of cells	104	106	107	106		
% cytotoxicity	9.62	10	11	8.5	9.92	1.2
	25 µg /ml					
Live cells	104	104	104	104		
Dead cells	7	7	9	7		
Total no. of cells	111	111	113	111		
% cytotoxicity	6.31	6.3	8	6.3	6.72	0.8
	12.5 µg /ml					
Live cells	107	107	107	107		
Dead cells	6	5	6	6		
Total no. of cells	113	112	113	113		
% cytotoxicity	5.31	4.5	5.3	5.3	5.1	0.4

Table 2: Result of *in vitro* cytotoxicity of *A. latifolia* using DLA cell lines

Drug concentration (µg/ml)	% Cell Death
12.5	5.1±0.4
25	6.72+0.8
50	9.92+1.2
100	25.1+0.8
200	43.7+2

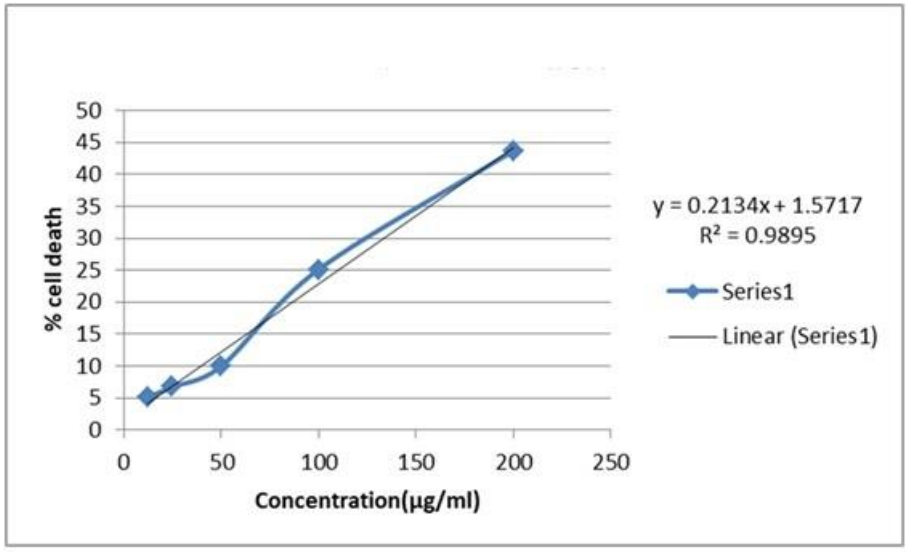

Fig. 1: *In vitro* cytotoxicity of *A. latifolia* extract using DLA cell lines. IC_{50} value is calculated by using the given equation in the graph, Y=MX+C Y=50, M=0.2134, C=1.5717, X is the IC_{50} value, so the equation is X=(Y-C)/M IC_{50} of the sample *A. latifolia* =226.9367

Tamilarasi *et al*. (2000) performed phyto-chemical and pharmacological evaluation of *A. latifolia.* The extracts were found to be safe up to a maximum dose of 500 mg/kg. There was no mortality and no changes were observed in behavioral neurological and autonomic responses. The present study indicated that the extract of *A. latifolia* is effective in inhibiting the growth of DLA cells at a concentration of approximately 226.94 µg/ml.

Investigation of in vitro antiproliferative activity of *A. latifolia* root extract on MCF-7 breast cancer cells was carried out by Viswanathan *et al*. (2023) . The IC_{50} concentration was found to be 62.5 mcg/ml and the cell viability was found to be dose dependent. The present study also revealed a dose-dependent cytotoxic effect of *A. latifolia* extracts on cancer cells.

Conclusion

This study aimed to investigate the *in vitro* cytotoxic effect of *A. latifolia*. The cytotoxicity studies revealed a dose-dependent cytotoxic effect of *A. latifolia* extracts on cancer cells. An IC_{50} value of 226.9367 indicates that the extract of *A. latifolia* is effective in inhibiting the growth of DLA cells at a concentration of approximately 226.94 µg/ml.

The findings of this study contribute to the growing body of knowledge on the pharmaco-logical properties of *A. latifolia* and provide a foundation for future research aimed at exploring its therapeutic potential in the development of

novel antimicrobial and anticancer drugs.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Botany, Carmel College (Autonomous) (Affiliated to the University of Calicut), Mala, Thrissur, Thrissur.

Author Contributions

Both the authors individually contributed in the experimental design, lab work, data analysis, manuscript writing etc. Authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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