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Research Article

Cytotoxic Effect Of *Dolichandrone Falcata* Against Human Cancer Cells- An In Vitro Approach

Roopa Kulkarni[1] and Paramjyothi Swamy[2*]

[1]Research Scholar, Department of Post Graduate Studies and Research in Biochemistry, Gulbarga University, Kalaburagi, Karnataka, India.
[2*]Professor, Department of Post Graduate Studies and Research in Biochemistry, Gulbarga University, Kalaburagi Karnataka, India.

***Corresponding Author:** Dr. Paramjyothi Swamy
*Professor, Department of Post Graduate Studies and Research in Biochemistry, Gulbarga University, Kalaburagi-585 106. Karnataka, India. Email: paramjyothiswamy@gmail.com

Abstract
In vitro anticancer activity of *Dolichandrone falcata* bark was studied using HEPG1 and MCF7 cell lines. Methanol extract of selected plant was prepared using soxlet apparatus. Preliminary phytochemical studies revealed presence of phenols , flavonoides, glycosides, and steroides . *In vitro* anticancer activity of *Dolichandrone falcata* bark methanol extract at various concentration (12.5-250g) was studied against the chosen cell lines using MTT assay by analysing the IC50. The test extract showed moderate toxicity with IC50 concentration of 269g/ml against HEPG2 cells and about 78g/ml against MCF7 cell lines.

Key words- *In vitro* anticancer , methanol extract , cell lines, MTT assay

***Author of Correspondence: Email:** paramjyothiswamy@gmail.com

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INTRODUCTION

Cancer is a group of diseases characterized by abnormal cell growth. This disease has been the leading cause of death worldwide; breast cancer is one of the most common causes of death in the world (1). This disease is caused by environmental and genetic factors. Both factors can spur cancer cells to proliferate or multiply cells and also prevent programmed cell death or apoptosis. Proliferation will generally spur the development of cancer cells to be massive (2). To stop the proliferation of cancer cells, chemotherapy drugs such as cisplatin, tamoxifen, and doxorubicin are commonly used (3). Some chemotherapeutic agents have high toxicity so that they can interfere with or damage normal tissues and cause resistance. Therefore, it is necessary to develop safer therapeutic agents (4).

Phytochemical compounds in plant extracts have a role as antioxidants that can be used in cancer therapy with minimal side effects (5).

Cell lines are extensively used in many aspects of laboratory research and mostly as in-vitro models in cancer research. They have a number of advantages; for example they are easy to handle and represent an unlimited self replicating source that can be grown in almost infinite quantities(6). In addition, they exhibit a relatively high degree of homogeneity and are easily replaced from frozen stocks if lost through

contamination. Studies with cell lines can reuse as an initial screen for agents that might regulate drug resistance(7,8). To establish more appropriate models of drug resistance and explore the differences that exist in the different drug resistant sublines selected by different treatments(9,10).

Dolichandrone falcata seem belong to family Bignoniaceae, Malawi. Tanzania, South Africa, and India are all home to the vine Rajputana. Bundelkhand, Bihar, Deccan, Mysore, and Maharashtra are among the Indian states where it can be found. The plant is mainly used for diabetes, anti-inflammatory, anxiolytic, analgesic, antiestrogenic, antimicrobial, antinociceptive, antibacterial, and immunomodulatory activities. The leaves are also used in the treatment of leukaemia and menorrhagia. *Dolichandrone falcata seem* contains alkaloids, carbohydrates, saponin, phenol, terpenoids, cardiac glycosides, steroids, and amino acids(11,12).

Present study was undertaken to evaluate anticancer potential of bark of this plant extracted with three different solvents.

MATERIALS AND METHODS

Collection and authentication of plant material

Dolichandrone falcata seem plant was collected from the Gulbarga university campus, Kalaburagi, Karnataka India. The taxonomic identity was confirmed through Department of Botany, Gulbarga University.Kalaburagi (Voucher specimen no GCAK-117)

Solvent extraction

The bark of *Dolichandrone falcata* seem was cleaned and shade dried at room temperature and finely powdered in the domestic mixer. 30gm of coarsely powdered sample was subjected to Soxhlet extraction with different solvents like methanol, chloroform, and petroleum ether. The solvents were evaporated to obtain semi-solid masses, which were further used in the study.

Preliminary photochemical screening

Photochemical analysis of methanol, chloroform, and pet ether extracts of bark of *Dolichandrone falcata* seem plant were carried out using the procedures as described by Trease, and Evans, Harborne, Soni and Sosa.

Cell lines and culture medium

Cell lines and culture medium The human breast cancer cell line (MCF7)and human hepatocellular carcinoma cell line (HEPG2) were parclated from National Center for Cell Sciences (NCCS), Pune and culture was maintained in Dulbecca's modified Eagles minimum with high glucose medium essential medium 10% Fetal Bovine Serum (FBS). All cells were maintained at 37^0C,in 5% CO2,atmospheric 95% air and 100% relative humidity.

Cell line morphological changes after giving *Dolichandrone falcata* bark extract

The cell lines MCF7 and HepG2 were put in to 6-well plates ($3ml\times10^5$cells /ml)and incubated for 24hrs. The cells were treated with *Dolichandrone falcata* bark extract at concentrations of 12.5,25,50,100 and 200µg/ml. After treatment the cell morphology was observed using inverted biological microscope.

Anticancer avtivity by MTT assay

In vitro anticancer activity of *Dolichandrone falcata* bark extract against MCF7 and HepG2 was carried out by MTT assay. 200ml of cell suspension (20,000 cells /well) was seeded in to 96 well disc plate and allowed to grow for 24hr. Test samples are added at concentrations of 12.5,25,50,100 and 200µg/ml. The plates were incubated for 24hrs at 37ºCin 5% CO2 atmosphere. After incubation period the spent media of treated cells was removed and the cultures were incubated with 0.5µg/ml of MTT at 37ºC for 3hrs.

Mitochondria that are active in living cells will reduce MTT to purple blue formazon crystals. The number of surviving cells is assumed to be according to the number of purple blue formazon crystals formed. After incubation the supernatent was taken and 100µl of DMSO was added to dissolve the purple blue formazon crystals.

The absorbance was calculated using an ELISA plate reader at 570nm. Percentage of cell viability was calculated using the formula, % cell viability is calculated using the below formula: % cell viability = [OD of treated cells/OD of Untreated cells] * 100 The IC_{50} value was determined by using linear regression The dose of test solution that reduces survival by half maximal inhibitory zone. Camptothecin was used as a standard control while test samples was used as a negative control. The cytotoxic activity was expressed with IC50 values obtained through a linear regression analysis between the logarithem of the concentration of test material and the percentage of cell viability(Table 3).

RESULTS AND DISCUSSION

The preliminary phytochemical screening of methanol extract of *Dolichandrone falcata* bark revealed presence of phenols , flavonoides glycosides and steroids. (Table 1). The effect of methanol extract and camptothecin (standard) on the growth of HepG2 and MCF7 cell lines was examined by MTT assay which is used to asses the number of viable cells and has been adopted to measure the growth modulation of cells in vitro. Dose dependent response was obtained between the range of 12.5200mg/ml decreasing number of viable cells with increasing concentration of extract.

Table1- Phytochemical constituents of *Dolichandrone falcata* bark

Sl, No	Phytochemicals	Methanol extract	Chloroform extract	Pet Ether extract
1	Alkaloides	+++	++	++
2	Carbohydrates	++	____	____
3	Cardiac glycosides	++	+	+
4	Flavonoides	+++	+++	++
6	Amino Acids	_____	_____	_____
7	Saponins	+++	_____	_____
8	Tannins	+++	+++	+++
9	Terpenoides	++	+	+
10	Quinones	+++	++	+

Maximum cytotoxic effect was noticed at 200µg/ml of test sample. That is 57.65% and 21.32% viability for HepG2 and MCF7 cell lines respectivelly. The extract showed an inhibitory concentration (IC50)of 269µg/ml for HepG2 cell line and 78µg/ml for MCF7 cell line. These results are in accordance with Rahayu et al(2023) who have reported cytotoxicity of Diellenia suffruticasa leaf extract against MCF7 and HepG2 cell lines using MTT assay stating it as a candidate for breast and liver cancer treatment. Similarly Syafruddin et al (2021) have reported anticancer property of methanolic extract of S, trilobata leaves against MCF7 breast cancer cell lines.

Table 2: In vitro cytotoxic activity of *Dolichandrone falcata* extract on HepG2 cell lines

Treatment Groups (µg/ml)	Cell Viability(%)
Blank	------
12.5	98.21
25	97.23
50	91.56
100	89.95
200	57.65
Standard	69.12

Table 3: In vitro cytotoxic activity of *Dolichandrone falcata* extract on MCF7 cell lines

Treatment Groups(µg/ml)	Cell Viability(%)
Blank	--------
12.5	98.56
25	85.96
50	60.20
100	40.52
200	21.32

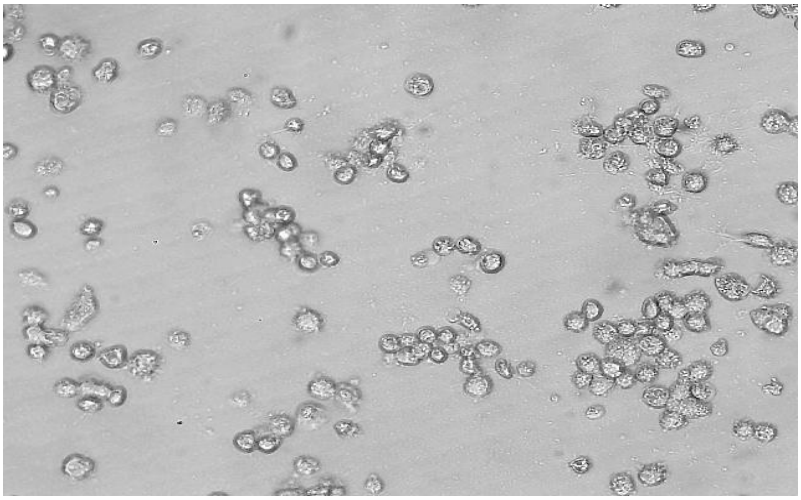


Figure 1- Histomorphological features of MCF7 cell lines untreated

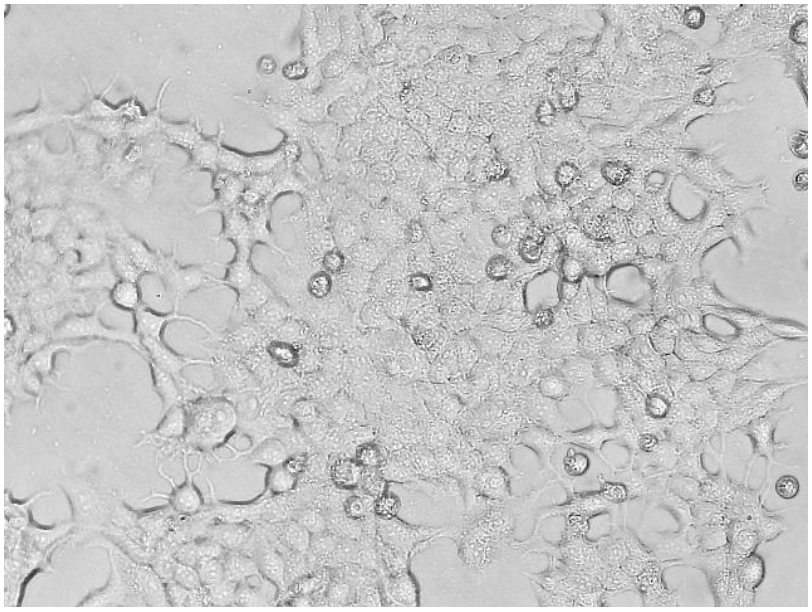


Figure2- Histomorphological features of MCF7 cell lines treated with standard drug

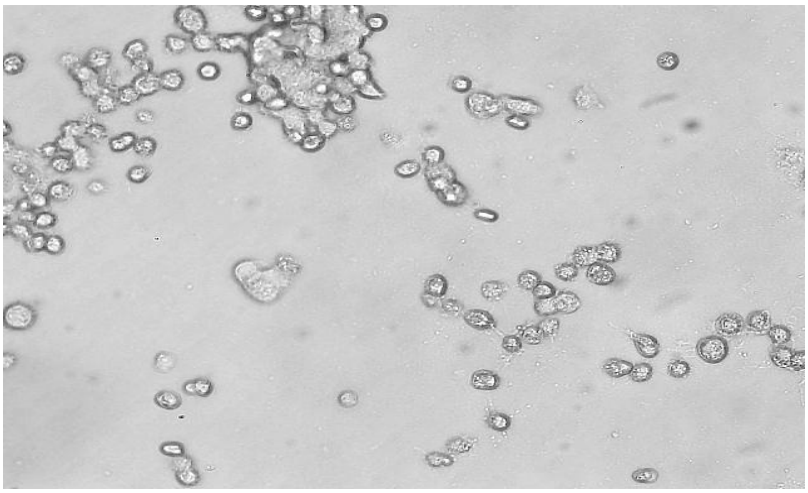


Figure 3- Histomorphological features of MCF7 cell lines treated with *Dolichandrone falcata* plant bark methanol extract

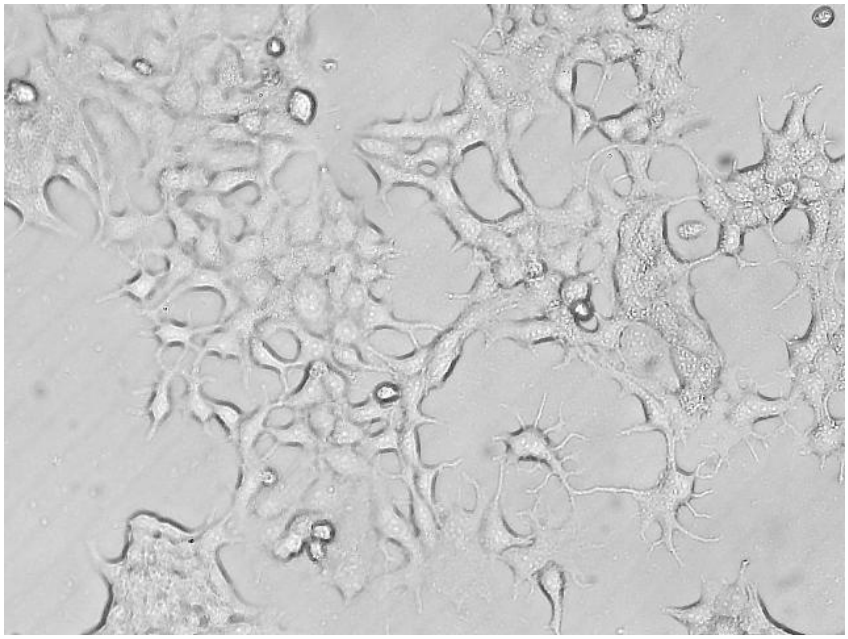


Figure 4- Histomorphological features of HepG2 cell lines untreated

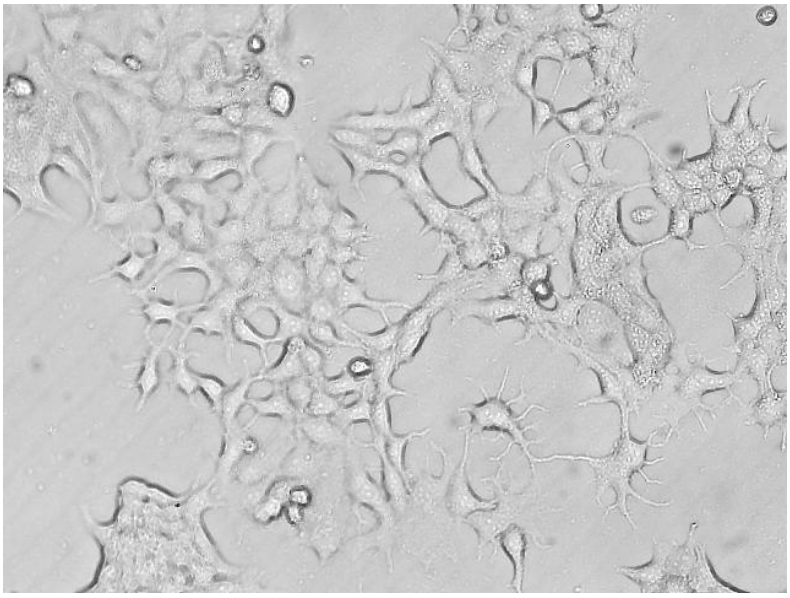


Figure 5- Histomorphological features of HepG2 cell lines treated with standard



Figure 6- Histomorphological features of HepG2 cell lines treated with *Dolichandrone falcata* plant bark methanol extract

Statastical analysis

To evaluate anticancer activity of the extract two human cancer cell lines MCF7 and HepG2 were exposed to a culture medium without or withtest extracts for 72hrs at 370C. Changes in morphology of cancer cells were monitored and analysed using inverted microscopy.

Further morphological differences were demonstrated as shown in fig (1 to 6) The cells were observed to experience a shrinkage and lysis, indicating the inhibited cellular growth.This appearance can be associated with the characteristics of cell cell death, where nuclear condensation occurs resulting in the formation of apoptotic bodies. Apoptis and necrocis are two major events leading to cell death (Nosrati et al 2021) , armania et al (2013) have reported about is important extract inducing breast cancer cell death undergoing apoptic sigaling Apoptic cells can be distinguished by their unique morphological changes from other cells. I e shrinkage of cells chromatin condensation plasma membrane blebbing and formation of apoptic bodies(17,18).

Antioxident activities are important for anticancer mechanism , at cancer initiation and development are strongly correlated with ROS, which in turn may be due to the presence of polyphenols, flavonoides, lignins, and xanthones(19,20).

CONCLUSION

Methanol extract of *Dolichandrone falcata* bark was found to be cytotoxic towards HepG2 and MCF7 cell lines in MTT assay, Dolichandrone falcata may be a chemotherapeutic / chemoprecentive agent based on its ability to induce apoptosis in cancer cells with relatively low toxic to normal cells.

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