

**IN-VITRO CYTOTOXIC ACTIVITY ON ROOTS OF
Clerodendrum phlomidis AGAINST NIH 3T3 CELL LINE
AND HELA CELL LINE**

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Summary

In this study, cytotoxic activity of *Clerodendrum phlomidis* crude extracts was investigated. Petroleum ether, chloroform, ethyl acetate and ethanol extracts prepared from roots of *Clerodendrum phlomidis* were tested for cytotoxic activity on Mouse embryonic fibroblasts cell line (NIH 3T3) and human cervical cancer cell line (HeLa) using MTT assay. The cells are tested at different concentrations to determine IC₅₀ (50% growth inhibition) by MTT assay. Chloroform extract shows moderate cytotoxic activity with an IC₅₀ value of 148.4 µg/ml on (NIH 3T3), while Pet ether and ethyl acetate have weak cytotoxic activity on (NIH 3T3). Ethanol extract has no cytotoxic effect on both NIH 3T3 and HeLa cell line. Ethyl acetate extract has moderate cytotoxic activity with an IC₅₀ 166µg/ml on HeLa cell line, while pet ether and chloroform have weak cytotoxic activity on HeLa cell line.

Keywords: *Clerodendrum phlomidis*; cytotoxic activity; crude extracts; MTT.

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Introduction

Clerodendrum phlomidis is a common shrub of arid plains, low hills and tropical deserts. They are distributed throughout the drier parts of India (Andhrapradesh, Uttarpradesh, Delhi, Gujarat, and Tamilnadu). *Clerodendrum phlomids* Linn, (Syn. *Clerodendrum multiflorum* (Burn.f) O.Kuntze, *Volkameria multiflorum* (Burn.f). (Verbenaceae) is an important and well known medicinal plant extensively used in Ayurveda and Siddha system of medicine for treatment of various ailments. The popular therapies include on inflammation, diabetic, nervous disorders, asthma, rheumatism, digestive disorders, and urinary disorders. It has been reported that pectolinarigenin, scutellarein, 7 hydroxy flavone has been isolated from this plant.¹ Decoction of its root which is slightly aromatic and astringent is used as a demulcent in gonorrhea. It is also given to children during convalescence from measles.² Roots contain clerodin, clerodendrin A, clerosterol, ceryl alcohol. The ethanol extract of leaves exhibited hepatoprotective activity. The aqueous extract of leaves exhibited in vitro anthelmintic activity. The plant also exhibited anti diabetic activity.^{3,4} The methanolic extract *Clerodendrum phlomidis* Linn shows minor tranquilizing property.⁵ It is used in amrit nectar tablets (Amrit nectar tablets containing 38 herbs.) The effect of aqueous and alcoholic extract of Amrit nectar tablet on rat liver microsomal lipid peroxidation is good.⁶ Methanolic extract of leaves of *Clerodendrum phlomidis* shows significant inhibitory against castor oil induced diarrhoea. The extract also showed a significant reduction in charcoal meal test in rats.⁷ Ethyl acetate and hexane extracts of leaves and stems of *Clerodendrum phlomidis* showed antifungal activity against plant and human pathogens.⁸

Present investigation was aimed to assess the cytotoxic potential of various extracts from roots of *Clerodendrum phlomidis* on NIH 3T3 and HeLa cell lines using MTT assay.

Materials and Methods

Collection and Identification of *Clerodendrum phlomidis*

The roots of *Clerodendrum phlomidis*, were collected from Chennai, Tamil Nadu, India. The plant material was identified by Dr.Sasikala Ethirajulu, Research officer, CCRAS, Govt.of India, Chennai. The roots of *Clerodendrum phlomidis* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The dried powder of the roots was extracted sequentially by hot continuous percolation method using Soxhlet apparatus⁹ using different polarities of solvents like petroleum ether, chloroform, ethyl acetate and ethanol. The dried root powder was packed in Soxhlet apparatus and successively extracted with petroleum ether by for 24 hrs. Then the marc was subjected to chloroform for 24 hrs, and the marc was subjected to ethyl acetate for 24 hrs and then marc was subjected to ethanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Cell Culture

The mouse embryonic fibroblasts cell line (NIH 3T3) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Dulbeccos modicied Eagles Minimum (DMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of 1×10^5 cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. After 24 hr the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 1000, 500, 250, 125 and 62.5 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 37°C, 5% CO₂,

95% air and 100% relative humidity for 48hr. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells¹⁰⁻¹³. After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using following formula.

$$\% \text{ cell Inhibition} = 100 - \text{Abs (sample)}/\text{Abs (control)} \times 100.$$

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC₅₀ was determined using Graph Pad Prism software.

Results and Discussion

As shown in Table 1. The petroleum ether extract of *Clerodendrum phlomidis* exhibits weaker cytotoxic effect on NIH 3T3 and HeLa cell lines with an IC₅₀ value of 213.6µg/ml and 264.2 µg/ml respectively. Pet ether extract on NIH 3T3 and HeLa at 500 µg have 92.63% and 100% of cell growth inhibition. And a regression value of 0.9976(R²) and 0.9898(R²) respectively.

Table 1: Determination of Cytotoxicity of Pet ether extract on NIH 3T3 and HeLa cell line

Extract	Conc µg/ml	NIH 3T3		R ²	HeLa		R ²
		% Cell Inhibition	IC ₅₀ µg/ml		% Cell Inhibition	IC ₅₀ µg/ml	
Pet ether	62.5	3.2753	213.6	0.9976	5.7631	264.2	0.9898
	125	10.4401			8.6130		
	250	65.4043			41.925		
	500	92.6305			100		
	1000	100			100		

As shown in Table 2. The chloroform extract *Clerodendrum phlomidis* on NIH 3T3 at 250 µg has 93.03% of cell growth inhibition. Chloroform extract exhibits moderate cytotoxic effect on NIH 3T3 with an IC₅₀ value of 148.4 µg/ml, and exhibits weak cytotoxic effect on HeLa cells with an IC₅₀ value of 199 µg/ml. Chloroform extract on NIH 3T3 and HeLa cell line have a regression value of 0.9993 (R²) and 0.9892 (R²) respectively.

Table 2: Determination of Cytotoxicity of Chloroform extract on NIH 3T3 and HeLa cell line

Extract	Conc µg/ml	NIH 3T3		R ²	HeLa		R ²
		% Cell Inhibition	IC ₅₀ µg/ml		% Cell Inhibition	IC ₅₀ µg/ml	
Chloroform	62.5	3.8894	148.4	0.9993	6.7131	199	0.9892
	125	30.0921			23.3058		
	250	93.0399			61.3679		
	500	100			500		
	1000	100			500		

As shown in Table 3. The ethyl acetate extract of *Clerodendrum phlomidis* has weak cytotoxic effect on NIH 3T3 with an IC₅₀ value of 210.2 µg/ml, and a moderate cytotoxic effect on HeLa cells with an IC₅₀ value of 164.2 µg/ml and cell growth inhibition of 78.78% at 250 µg. Ethyl acetate extract on NIH 3T3 and HeLa cell line have a regression value of 0.9849 (R²) and 0.9967 (R²) respectively.

Table 3: Determination of Cytotoxicity of Ethyl acetate extract on NIH 3T3 and HeLa cell line

Extract	Conc µg/ml	NIH 3T3		R ²	HeLa		R ²
		% Cell Inhibition	IC ₅₀ µg/ml		% Cell Inhibition	IC ₅₀ µg/ml	
Ethyl acetate	62.5	3.3776	210.2	0.9849	8.2963	164.2	0.9967
	125	23.2343			28.7523		
	250	56.3971			78.7840		
	500	100			100		
	1000	100			100		

As shown in Table 4. The ethanol extract of *Clerodendrum phlomidis* has no cytotoxic effect on NIH 3T3 and HeLa cell line with an IC₅₀ value of 537.8 µg/ml and 486.9 µg/ml respectively. Ethanol extract on NIH 3T3 and HeLa cell line have a regression value of 0.9892 (R²) and 0.9894 (R²) respectively.

Table 4: Determination of Cytotoxicity of Ethanol extract on NIH 3T3 and HeLa cell line

Extract	Conc µg/ml	NIH 3T3		R ²	HeLa		R ²
		% Cell Inhibition	IC ₅₀ µg/ml		% Cell Inhibition	IC ₅₀ µg/ml	
Ethanol	62.5	2.96827	537.8	0.9892	2.0899	486.9	0.9894
	125	8.9048			12.9829		
	250	29.6827			22.4825		
	500	45.0358			48.2583		
	1000	71.0337			81.4439		

Conclusion

The above mentioned results suggest that chloroform extract of *Clerodendrum phlomidis* has moderate cytotoxic effect on NIH 3T3 cell line. Ethyl acetate extract has moderate cytotoxic activity on HeLa cell line. Ethanol has no cytotoxic activity on both the cells. Future study is needed to isolate the active constituent and subject to pharmacological screening of this plant.

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