



In Vitro Anticancer Activity of Ethanolic Extract of *Clerodendron infortunatum* Linn. Leaf

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ABSTRACT

Medicinal plants have been traditionally used for preventing and curing a number of diseases, mostly in India. *Clerodendron infortunatum* Linn. widely reported to have pharmacological uses arising from its wide- spread folkloric uses. The plant is belongs to the family Verbenaceae. In Hindi, it is known as Bhanti. Traditionally the plant is used in the Siddha system of medicine. It is one of the main ingredients of many formulations for the treatment of rheumatic conditions, diabetes, diarrhoea, and fever, skin ailments and treating wounds. The present study has been proposed to evaluate the anticancer activity of the ethanolic extract of leaf part. A stock solution of the 1mg/ml was prepared. From this solution in a final concentration of 6.25 µg/ml, 12.5µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml were prepared. These solutions were added on L929 (Fibroblast cell) cell lines for the in-vitro anti cancer activity. The observation of the study detailed that, ethanolic extract of *Clerodendron infortunatum* Linn. Possesses remarkable anticancer activity on the tested cell lines and is increased as the concentration of drug extracts increases.

Keywords: Clerodendron, Anticancer, Cellline, Fibroblast, Extract.

INTRODUCTION

Clerodendron infortunatum Linn. is a shrub grow in up to 2-4 ft height, belongs to the family Verbeaceae,¹ wide spread in the south, eastern regions and found along the sides of road all over in India. The species is native to tropical regions of Myanmar, Pakistan, Thailand and Sri Lanka. The shrub has been identified by Linnaeus in 1753. More than 500 species of the genus *Clerodendrum* are identified today and the Phytomedical importance of this different species of the plant genus has been reported in various indigenous systems of medicines. The plant flowers during the period February- May (Haines, 1925. In Ayurveda it is known by the Sanskrit names "Bharhi", "Bhrigubhava", "Padma", "Fanji", and "Brahman yastika" (Shastri,1977). In Kerela, as "Peruvelum," and in Hindi as "Bhant" or "Bharangi".^{2,3}

Morphology⁶

The plant is a flowering small tree or shrub. Its stem is erect, hollow with 0.5-4m height. The leaves are simple, almost circular opposite; diameter- 6 inch. It is sparsely villous-pubescent, elliptic, broadly elliptic, and ovate or elongate ovate, 3.5–20 cm wide, 6–25 cm long, dentate. The plant produces terminalin florescence and flowers. These are white with purplish pink or dull-purple throat, pubescent. Berry fruit, globose and turned bluish-black or black when it is ripe. The fruit is enclosed in the red accrescent fruiting-calyx. Fruit usually with 4 dry nutlets and the seeds may be with or without endosperm.¹¹

Phytochemical constituents¹¹

Reported phytochemical constituents of the leaves are,

saponin - clerodin (abitter diterpene), b. Enzymes, c. alkyl sterols, d. 2,-(3, 4-dehydroxyphenyl)ethanol-1-O-α-2 rhamnopyranosyl (1→3)-β-D-(4-Ocaffeoyl), e. Fixed oil consist of glycerides of lenoleic, oleic, stearic and lignoceric acid. f. Phenolics-Acetiside, methyl and ethyl esters of caffeic acid, fumaric acid. g. Flavonoids-apegenin, acacetin, methyl esters of acacetin7-o-glucoronode. quercetin, hispidulin. h. steroids-clerodolone, clerodone, clerodol and clerosterol. i. sugars- glucose, fructose, raffinose, lactose, maltose.

MATERIALS ND METHODS

The chemicals and reagents required for the study were purchased from approved suppliers and were used as supplied.

Collection of plant material forthe study

The plant material was harvested and collected from central kerala region of south India. Leaves of the plant were separated, cleaned and was dried by placed under sun shade for a period of two weeks. There after the dried product was taken for maceration procedures. Dried leaves were cut in to small pieces for maceration. The material was immersed in 95% ethyl alcohol for one week with occasional shaking Extract was separated out and concentrated by evaporation. A thick greenish black product was resulted.

Determination of anti-cancer activity on Cultured L929Cell lines.^{12, 13}

The cell line L929 (Fibroblast cells) were purchased from NCCS Pune. The cells were maintained in Dulbecco's

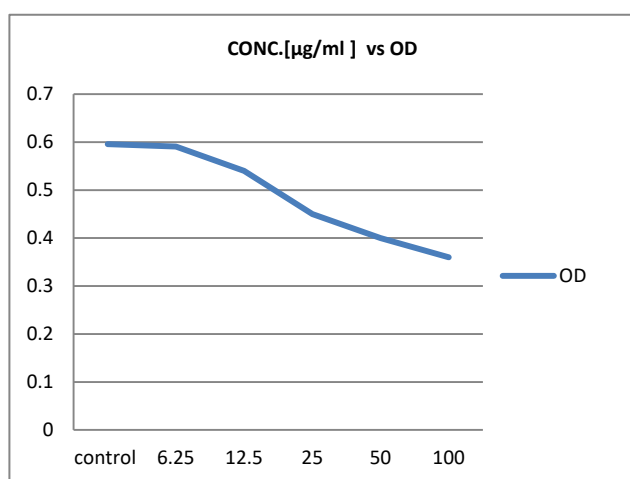


modified eagles media (HIMEDIA) containing 10% FBS (Invitrogen), and allowed to grow to confluence at 37°C in 5 % CO₂ in a humidified atmosphere in a CO₂ incubator (NBS, EPPENDORF, GERMANY). Then added 500 µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia) and treated for 2 minutes. The trypsinized cell material was transferred to T flasks in complete aseptic conditions. A stock of the extract in a concentration 1mg/ml was prepared and from this, different dilutions of 6.25, 12.5, 25, 50 and 100 µg/ml were prepared. These dilutions were added to the trypsinized cells and then incubated for 24hrs. The standard MTT assay was used for determining the percentage difference in viability, after 24 hours of incubation.

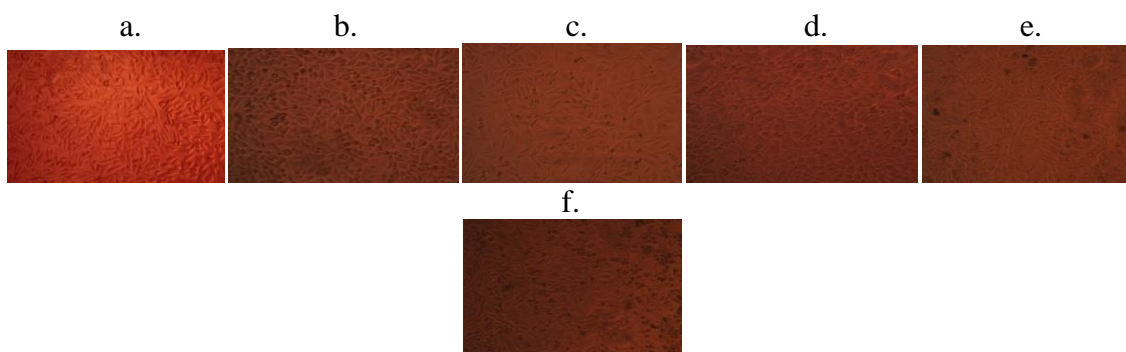
MTT ASSAY^{12, 13}

The incubated cells were washed with 1x PBS, added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS and then incubated at 37°C for 3 hours. After the incubation period the cells were washed to remove MTT with 1x PBS and added 200 µl of DMSO to the culture. The culture was incubated at room temperature for 30 minutes, until the cells lysed and the colour was produced. Centrifuged the solution at top speed for 2 minutes to separate out cell debris and the optical density was read at 540 nm using DMSO as blank in a microplate reader.

$$\% \text{ viability} = (\text{OD of Test} / \text{OD of Control}) \times 10$$



Name of material	OD Values (540nm)	Percentage Viability
Control (DMSO)	0.5957	100
Sample µg/ml		
6.25	0.5908	99.17744
12.5	0.5478	91.95904
25	0.4565	76.63253
50	0.4015	67.3997
100	0.3639	61.0878



Control 6.25µg/ml 12.5µg/ml 25 µg/ml 50 µg/ml 100 µg/ml

Summary

The medicinal herb *Clerodendron infortunatum* Linn is traditionally been reported to have its pharmacological activities, marked anti-inflammatory and antimicrobial. The ethanolic extract of the dried leaf part was studied for the anti-cancer activity on cultured cell line. As a result the study describes that change in concentration of drug extract changes the activity, and hence it is dose dependent. The highest concentration used for the test was 100 µg/ml, and is shown to reduce the cell growth to about 50%.

CONCLUSION

The *in vitro* anticancer activity of ethanolic extract of the leaves of *Clerodendron infortunatum* Linn. Demonstrates the photochemical constituent present in the plant extract possesses marked inhibitory activity on cancer cells and is seemed to be increased in higher concentrations. Though it is concluded that this study has to be followed through preclinical and clinical model for supportive and confirmation.

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