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V. Lakshmi,
Research Scholar,
Department of Botany,
Kundavai Naachiyaar Government Arts College For Women (Autonomous),
Thanjavur, Tamil Nadu, S. India

*Corresponding author
G. Viji Stella Bai
Department of Botany,
Kundavai Naachiyaar Government Arts College for Women (Autonomous),Thanjavur,
Tamil Nadu, S. India

Research Article **Botany**

In vitro anticancer activity of *Clerodendrum phlomidis* leaves and its silver nanoparticles on human breast cancer cell line (MCF-7)

V. Lakshmi[1] and G. Viji Stella Bai[2]

ABSTRACT

In the current study *Clerodendrum phlomidis* leaves and its silver nanoparticles were examined for their anticancer activity. To determine *in vitro* anticancer activity, different concentrations of *Clerodendrum phlomidis* leaves and its silver nanoparticles were tested on MCF- 7 cancer cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *Clerodendrum phlomidis* leaves and its silver nanoparticles showed a significant antiproliferative activity and a dose dependent effect was observed. The percentage of cell viability is 49 % was shown by extract at concentration 200 µg/ml and 82% was observed at 25 µg/ml. The percentage of cell viability is 29 % was shown by nanoparticle at concentration 200 µg/ml and 64% was observed at 25 µg/ml. The silver nanoparticles showed activity in potential anticancer activity.

Keywords: *Clerodendrum phlomidis*, Silver nanoparticle, MCF- 7 cancer cell line, MTT assay, anticancer activity.

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INTRODUCTION

Cancer is a much-feared disease in the modern society, and is one of the leading causes of mortality worldwide. It is a disease of genes; a disorder which occurs in the normal processes of cell division. Various carcinogens such as viruses, chemical carcinogens, and radiation cause aberration of the genetic material (DNA) of cells and ultimately the uncontrolled autonomous cell proliferation that defines cancer. Many human cancers are caused by exposure to food mutagens and carcinogens. Although complete elimination of daily exposure to dietary carcinogens may not always be possible, chemoprevention is an alternative approach to decreasing the carcinogenic effect. Natural chemopreventive agents have exhibited inhibitory effects on the initiation, promotion, and progression stages in carcinogenesis (Wattenberg, 1985) and

these agents are present in our diet (Wattenberg, 1992). With up to 60% of all cancers being related to dietary factors, diet and nutrition are perhaps the most important aspects of any cancer prevention program.

MCF-7 (Michigan Cancer Foundation-7 (Breast cancer cell) is a cell line that was first isolated in 1970 from the breast tissue of a 69-year old Caucasian woman. Of the two mastectomies she received, the first revealed the removed tissue to be benign. Five years later, a second operation revealed malignant adenocarcinoma in a pleural effusion from which was taken cells for MCF-7. The woman was treated for breast cancer with radiotheraphy and hormonotherapy. MCF-7 is a human breast adenocarcinoma cell line and has been used extensively in research; these cells were used in seminal studies on the estrogen receptor. A xenograft mouse model of MCF-7 breast cancer is used in studies to monitor cancer progression (MCF7 Xenograft Models). MCF 7 cells are cultured normally until they are confluent, but are then surgically implanted into a mouse's mammary fat pad. Tumor size can then be assessed as the disease progresses (Soule *et al*., 1973). MCF-7 cells are useful for *in vitro* breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen, in the form of estradiol, via estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor (ER) positive control cell line (Mosmann, 1983). In addition to retaining their estrogen sensitivity, MCF-7 cells are also sensitive to cytokeratin. They are unreceptive to desmin, endothelin, GAP, and vimentin. When grown in *in vitro*, the cell line is capable of forming domes and the epithelial like cells grow in monolayers. Growth can be inhibited using tumor necrosis factor alpha (TNF alpha), and treatment of MCF-7 cancer cells with anti-estrogens can modulate insulin-like growth factor finding protein's, which ultimately have the effect of a reduction in cell growth (Skehan *et al*., 1990).

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. (Uniyal *et al.*,2003). Plants used for treating various ailments of both man and animal are as old practice as man himself. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Ayurveda, Siddha and Unani) (Dahanukar *et al.*, 2000). The medicinal value of the chosen plant *Clerodendrum phlomidis* leaves has not been extensively worked out. Therefore, the present study was to examine the *in vitro* anticancer activity of *Clerodendrum phlomidis* L.leaves and its silver nanoparticles tested against MCF-7 cell line.

MATERIALS AND METHODS

Cell line and Culture

Breast cancer- MCF-7 cell lines was obtained from National centre for cell sciences, Pune (NCCS). The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories FBS was purchased from Cistron laboratories Trypsin, 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl- tetrazolium bromide (MTT) and Dimethyl Sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals, Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

Cytotoxicity assay

The viability of cells was assessed by MTT assay (Mosmann, 1983) using MCF-7 cell line. The cells were plated separately in 96 well plates at a concentration of 1×10^5 cells/well. After 24 h, cells were washed twice with 100 µl of serum-free medium and starved for an hour at $37^{o.C}$. After starvation, cells were treated with the test material for 24 h. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37ºC in a CO_2 incubator. The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by pipetting up and down. Spectrophotometrical absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graph pad prism5 software

RESULTS

IN VITRO ANTICANCER ACTIVITY

The cytotoxic effect of the biosynthesized *Clerodendrum phlomidis* L. leaf extract and nanoparticles were examined on cultured MCF 7 Cells by exposing cells for 24 h to medium containing the plant extract and SNPs at 25 –200 µg/ml concentrations. The *Clerodendrumphlomidis* leaf extract and nanoparticles inhibited the growth of

the cancer cells extensively,in a dose dependent manner (Table 1, plate 1 &2 and Fig 1). The nanoparticles inhibited the growth of the cancer cells considerably as compared to *Clerodendrum phlomidis* L leaf extract.

Table 1: MTT assay using MCF-7 cell line.

Plant sample	Optical density		% of cell viability	
Control	1.39	1.39	100	100
25 µg	1.14	1.12	82.01439	80.57554
50 µg	1.01	1.05	72.66187	75.53957
100 µg	0.871	0.843	62.66187	60.64748
200 µg	0.694	0.701	49.92806	50.43165
Nanoparticle				
Control	1.39	1.39	100	100
25 µg	0.894	0.912	64.31655	65.61151
50 µg	0.601	0.644	43.23741	46.33094
100 µg	0.489	0.497	35.17986	35.7554
200 µg	0.411	0.4	29.56835	28.77698

Fig 1: % of Cell viability of *C. phlomidis* and nanoparticles

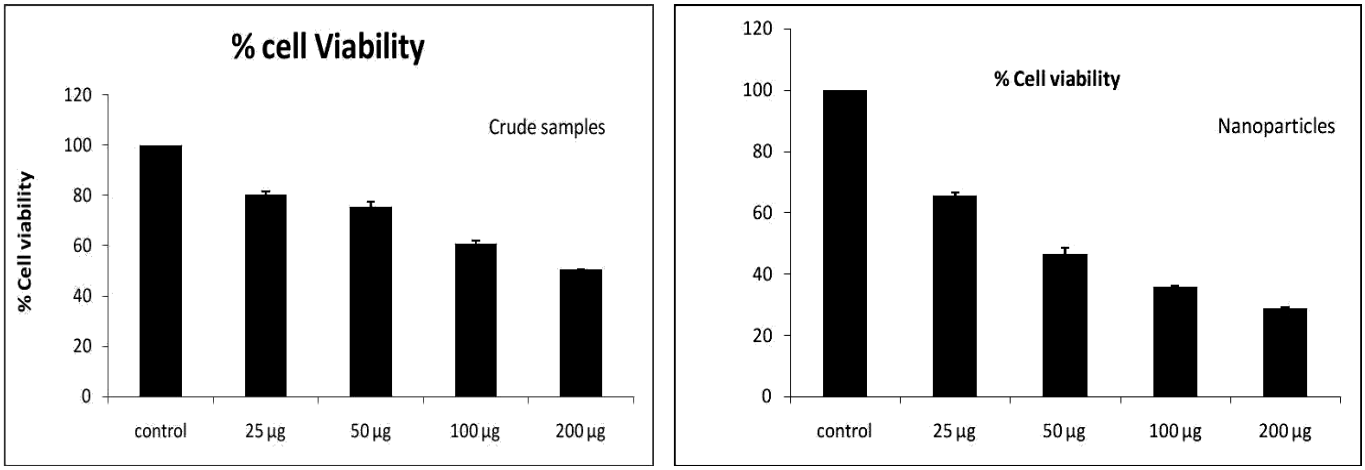
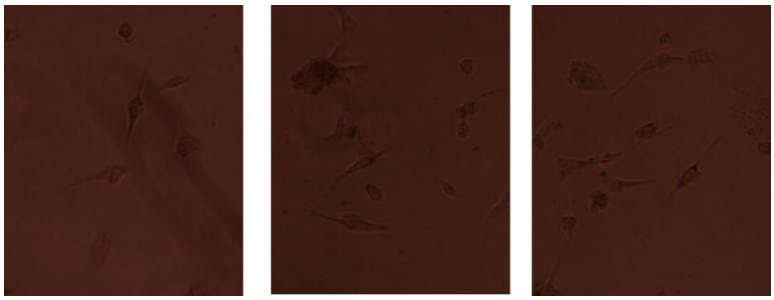


Plate 1- Cytotoxic assay of *Clerodendrum phlomidis* L. leaf

Control **25µg Plant extract treated** **50µg Plant extract treated**



100µg Plant extract treated **200µg Plant extract treated**

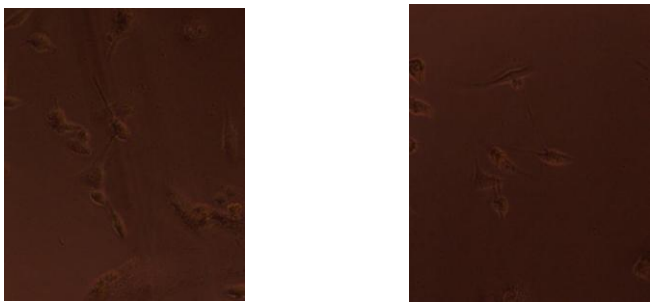
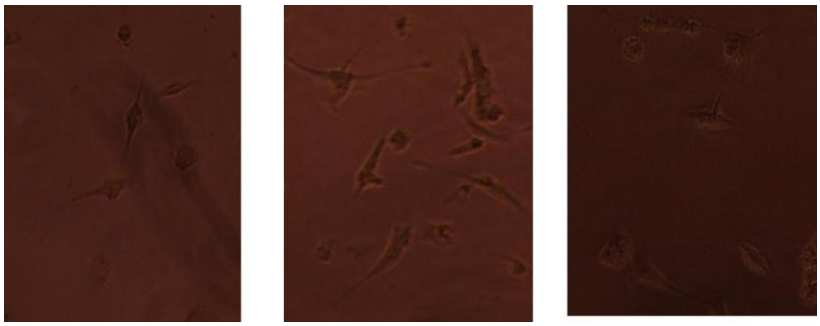
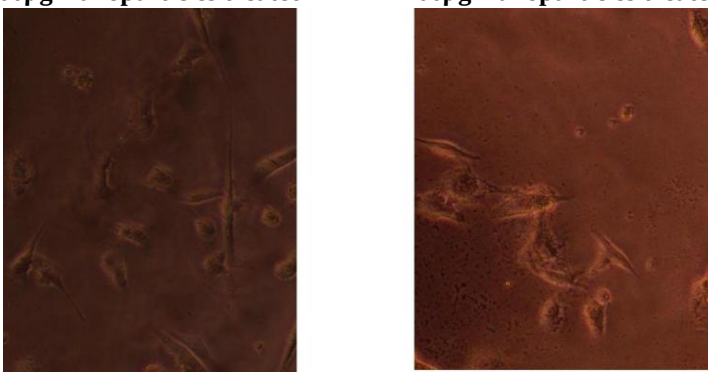


Plate 2- Cytotoxic assay of Nanoparticles

Control **l25µg Nanoparticles treated** **50µg Nanoparticles treated**



100µg Nanoparticles treated **200µg Nanoparticles treated**



DISCUSSION

The cytotoxic effect of the biosynthesized *Clerodendrum phlomidis* leaf extract and nanoparticleswere examined on cultured MCF 7 Cells by exposing cells for 24 h to medium containing the complex at 0.6 – 30 g/ml concentration. The *Clerodendrum phlomidis* L. leaf extract and nanoparticles inhibited the growth of the cancer cells significantly, in a dose and duration dependent manner. The cytotoxic effect of the sample may be interpretable as due to its amphiphilic nature and hence, would penetrate the cell membrane easily, reduce the energy status in tumors and also alter hypoxia status in the cancer cell micro environment, which are factors that would influence the antitumor acidity. It is known that biosynthesized Silver nanoparticles have a wide range of biological activities such as antitumor, antifungal, apoptosis, interaction with DNA thereby inhibiting replication, transcription, and other nuclear functions and arresting cancer cell proliferation so as to arrest tumor growth.

Sunita and Soumya (2012) investigated the cytotoxic potential of leaf and bark extracts of two *Clerodendrum* sps namely *Clerodendrum phlomidis* L. and *C.viscosum*. Solvent extracts hexane, chloroform, acetone and methanol were tested for their cytotoxic potential using brine shrimp motility assay. Cytotoxic activity of all the solvent extracts was tested at four doses 25, 50,100 and 200µl/ml. All the extracts showed dose dependent activity. Acetone extract of leaf (*C.viscosum*) showed significant cytotoxic activity 90.6 % at the dose of 200microgram/ml whereas chloroform extract of *C. phlomidis* (bark) showed highly significant activity to the tune of 95.6% at the highest dose.

Overall, it can be concluded from the present study that *Clerodendrum phlomidis* leaves extract and silver nanopartilce might be a potential alternative agent for human breast cancer therapy. The nanoparticles inhibited the growth of the cancer cells significant as compared to *Clerodendrum phlomidis* L leaf extract. Hence, it is anticipated that would be a useful pharmaceuticalmaterial to treat breast cancer.

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