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# *In-vitro* anti-cancer studies of Cinnamaldehyde on breast cancer cell line (MCF-7)

**Meena Vangalapati*, Sree Satya Nandam, D.V.Surya Prakash**

**Centre of Biotechnology, Department of Chemical Engineering, AUCE (A), Andhra University, Visakhapatnam 530003, (INDIA)**

**E-mail: meena_sekhar09@yahoo.co.in**

## ABSTRACT

Cinnamaldehyde occurs naturally in the bark of Cinnamon tress and other species of the genus *Cinnamomum* like Camphor and Cassia. Cinnamon bark contains Cinnamaldehyde about 80-90%. Cinnamaldehyde showed many bioactivities including inhibition of cancer cell growth, Antidiabetic, anti-fungal, anti-bacterial activities etc. The present work aims that *in-vitro* anti-cancer activity of Cinnamaldehyde on Breast cancer MCF-7 cell lines by using MTT cell growth inhibition assay. The highest percentage inhibition of cancer cell lines was found to be 32.3% at a dose of 200µg/ml.



## KEYWORDS

Cinnamon;
Cinnamaldehyde;
Anti-cancer activity;
MCF-7 cancer cells;
MTT assay.

## INTRODUCTION

Cancer is one of the major human diseases. In recent years the role of plants in our daily life is increased because of the beneficial effect in the prevention of human diseases[1] such as heart diseases, diabetes etc. Plants are used as tools in cancer research[2]. Because plants containing many number of phyto constituents. Some of the phyto constituents are flavonoids, quinones, and terpenoids. These are the abundant sources in our daily diet.

MCF-7 (breast cancer cell line) is one of the cancer cell lines. MCF-7 is the acronym of Michigan Cancer Foundation, where the cell line was established in 1973 by Hebert Soule and co-workers. 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.

Cinnamon is a spice which belongs to Lauraceae family which is originated from Sri Lanka, East and West India, Burma, Indonesia and Vietnam[3]. It is not only used as spice it has various medicinal properties such as antipyretic, antioxidant[4], antibacterial[5], antitermitic[6], antifungal[7], and anti-inflammatory[8]. Cinnamon bark contains much number of chemical constituents[9] like Cinnamaldehyde, Eugenol, Cinnamic acid, Cinnamyl acetate etc. Among those constituents the important compound is Cinnamaldehyde (3-phenyl-acrolein, 65 to 75%). The molecular formula for Cinnamaldehyde is $C_9H_8O$ and the molecular structure is shown in Figure 1. Cinnamaldehyde has been shown to inhibit proliferation of several human cancer cell lines including breast, leukemia, ovarian and lung tumor cells[10]. It has been shown various activities such as

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antitumor, antifungal, Cytotoxic and mutagenic[11,12]. In the present study, the effect of Cinnamaldehyde on Breast cancer MCF-7 cell lines by using MTT cell growth inhibition assay was studied.

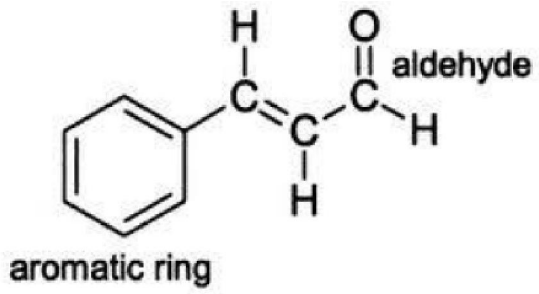


**Figure 1 : Cinnamaldehyde.**

## MATERIALS AND METHODS

### Materials

The dried bark of Cinnamon bark was collected from the local market Visakhapatnam, Andhra Pradesh. Clean the bark and dried under sunlight for 1 day. The dried bark was powdered and used as a raw material and stored in the air tight container. It is finely grounded to 120 mesh size.

### Chemicals

MCF-7 Cancer cell lines, 4.5 g/L glucose, 2 mM L-glutamine, 5% fetal bovine serum (FBS) Standard drug (Tamoxifen), MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), 1x phosphate buffer saline (1x PBS), Dimethyl Sulfoxide (DMSO).

### Preparation of extract

Cinnamaldehyde was extracted from 80% (v/v) methanolic extract[13] of Cinnamon from the Steam Distillation. The final extract from steam distillation[14] was collected and purified with hexane in 1:1 ratio. The purified sample of Cinnamaldehyde was used for anti-cancer studies on MCF-7 cell lines.

### Maintenance of MCF-7 (Breast Cancer) cell lines

MCF-7cell lines were procured from the National Centre for Cell Science, Pune. Cell lines were grown in Minimal essential medium (MEM) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at $37^0$C in 5% $CO_2$ incubator.

### MTT assay

The MTT assay developed by Mosmann[15] was modified and used to determine the inhibitory effects of test compounds on cell growth *in vitro*. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% $CO_2$ to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds (12.5, 25, 50, 100 and 200 µg/mL) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/mL concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5 µl of fresh MTT (0.5 mg/mL in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the coloured formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 492 nm on an ELISA reader, Anthos 2020 spectrophotometer.

The percentage inhibition of cancer cell lines can be calculated as

$$100-(A_t - A_b) / (A_c - A_b) \times 100$$

$A_t$ : Absorbance of test,
$A_b$ : Absorbance of blank,
$A_c$ : Absorbance of control.

## RESULTS AND DISCUSSION

The Cinnamaldehyde of Cinnamon species has showed significant activity at various concentrations and its effect was compared with the standard drug Tamoxifen. The maximum percentage inhibition of cancer cell lines was observed as 32.3% at 200µg/ml as shown in TABLE 1. From the Figure 1 it was found that the concentration of Cinnamaldehyde was increased from 12.5 to 200µg/mL and the % inhibition of MCF-7 cell lines was also increased from 4.5% to 32.3% that means Cinnamaldehyde induces a cell arrest to inhibit the growth of the MCF-7.

**TABLE 1 : Effect of Cinnamaldehyde and standard drug (Tamoxifen) on %Inhibition of MCF-7 cancer cell lines.**

| Concentration (µg/mL) | Absorbance of Tamoxifen at 492 nm | % Inhibition of cell lines by Tamoxifen | Absorbance of Cinnamaldehyde at 492 nm | % Inhibition of cell lines by Cinnamaldehyde |
|---|---|---|---|---|
| 12.5 | 0.468 | 10.7 | 0.728 | 4.5 |
| 25 | 0.366 | 34 | 0.713 | 6.7 |
| 50 | 0.201 | 66.4 | 0.584 | 24.5 |
| 100 | 0.126 | 82.1 | 0.553 | 28.8 |
| 200 | 0.110 | 85.4 | 0.528 | 32.3 |

**Blank: 0.040; Control: 0.761**

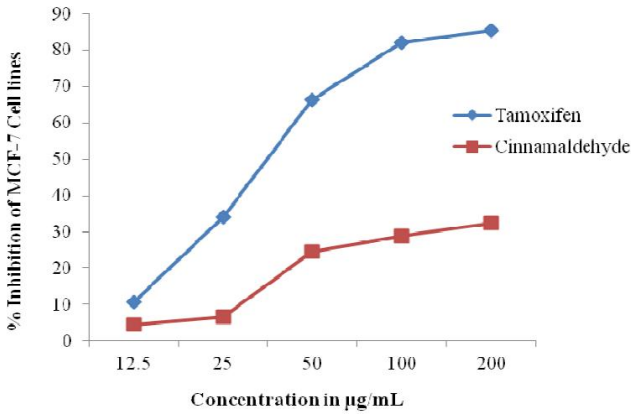


**Figure 2 : Effect of Cinnamaldehyde and standard drug (Tamoxifen) on %inhibition of MCF-7 cancer cell lines.**

## CONCLUSION

Cinnamaldehyde was a main compound in Cinnamon species. It shows the inhibition of cancer cell growth, contractile responses of cardiovascular muscles, anti-fungal, anti-bacterial activities etc. The anti-cancer studies of Cinnamaldehyde on Breast cancer cell lines (MCF-7) was carried out by using MTT cell growth inhibition assay. The results showed that the maximum percentage inhibition of cancer cell lines for Cinnamaldehyde was found to be 32.3% at a dose of 200µg/ml. So Cinnamaldehyde acts as an anti-cancer agent.

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