



# ANTICANCER ACTIVITY OF *TAMARINDUS INDICA* FRUIT PULP AND *CASSIA AURICULATA* LEAVES EXTRACT AGAINST BREAST CANCER CELL LINE

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## Abstract

Breast cancer is the most common cause of cancer in women and the second most a common cause of cancer death in women in the U.S. Breast cancer refers to cancers originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. This study was aimed to analyze that anticancer activity of *Tamarindus indica* L. fruit pulp and *Cassia auriculata* L. leaves extract against breast cancer cell line and evaluate Qualitative phytochemical analysis. Then evaluate Quantitative phytochemical analysis. Finally evaluate the DPPH assay. To evaluate the MTT assay. To evaluate DNA fragmentation. Phytochemical analysis various test analysed done and cell line are studied and viability are calculated. Molecular mechanisms of action of biologically active substances derived from plants are associated with cytotoxicity, cytostaticity and/or with apoptosis. Apoptosis (programmed cell death) is initiated in response to damages in hereditary material and represents a series of genetically controlled events, resulting in elimination of damaged cells.

**Key words:** Breast cancer, cytotoxicity, diseases, carcinoma

## Introduction

Plant use in treating diseases is as old as civilization (Alviano & Alviano, 2009; Amin & Mousa, 2007) and traditional medicines are still a major part of habitual treatments of different maladies (Anand & Kunnumakara, 2008; Arden & Betenbaugh, 2004). Cancer is a fatal disease that is caused when cells in the body grow or divided in an uncontrolled manner and not normal, killing normal cells and often causing death. Cancer is the second dreadful and very serious disease in the world. It causes about 13% of all annual deaths worldwide (Azaizeh *et al.*, 2006; Anooj *et al.*, 2019; Bedir & Khan, 2000).

Breast cancer is the most common cause of cancer in women and the second most a common cause of cancer death in women in the U.S. Breast cancer refers to cancers originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Worldwide, breast cancer comprises 10.4% of all cancer incidences among women,

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making it the second most common type of non-skin cancer (after lung cancer) and the fifth most common cause of cancer death. In 2004, breast cancer caused 519,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths). Breast cancer is about 100 times more common in women than in men, although males tend to have poorer outcomes due to delays in diagnosis (Lekshmi & Praseetha, 2019; Lekshmi *et al.*, 2019; Bedir *et al.*, 2002).

The MCF-7 breast cancer cell line originated from a 69-year-old Caucasian woman who previously underwent two mastectomies in a five-year span. The tissue removed at the first mastectomy was benign, but the second operation found a malignant adenocarcinoma. Over a period of three years, the woman was treated for local recurrences by radiotherapy and hormonotherapy (Bertino, 1997).

## Materials and methods

### Collection of Sample

Plants such as *Cassia auriculata* L. and

*Tamarindus indica* L. were identified and collected in and around the Vellore district in Tamil Nadu. Plants were dried under shade, powdered, extracted using solvent by extraction method using Whatmann No.1 filter paper. The extracts were collected, dried and stored in room temperature for future use.

### Sequential Extraction

Fresh leaves of *Cassia auriculata* and fresh fruit pulp of *Tamarindus indica* were rinsed thoroughly in running tap water, cut to tiny pieces and air dried at room temperature. The dried leaves and pulp, each was milled to fine powder. A 100g portion of the pulverized leaves and fruit pulp was extracted by maceration in 200ml of absolute hexane, ethylacetate and ethanol (BDH) kept for 24 hours Okoli *et al.*, (2005). Each extract was filtered through a Whatman No. 1 filter paper and the pH determined; and the filtrate was concentrated by evaporation to dryness in a steady air current for about 24 hr in a previously weighed Petri dish. After evaporation, the Petri dish and residue were reweighed and weight of the extract determined. All extracts were stored in sterile containers at room temperature until they were used.

### Phytochemical Analysis

In addition that, dried powdered samples was subjected to qualitative tests for the identified of phytochemical constituents according to standard procedure table 2 and 3. The preliminary phytochemical investigation showed the presence of Phytochemical constituents such as carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, steroids & phytosteroids, phlobatannins, anthraquinones. The fruit pulp *Tamarindus indica* L. and *Cassia auriculata* leaves was subjected to qualitative analytical tests for the various plant.

### In vitro Anticancer Activity (MTT Assay)

MCF-7 Cell line was taken from the organism and maintaining them in the lab. The Cell line was cultured in DMEM medium with 10% Foetal Bovine Serum+Antibiotic solution. The cell line was cultured humidified air (95%) at pH 7.1 cell require and nutrient medium temperature 37°C with the remaining 5% CO<sub>2</sub>. The cell line is divided into two types: Ethereal and Non-adhere. MCF-7 belongs to Ethereal cell line. The subculture is two division into Passage (T-flask) and Seeding (Microtitre plate).

### Cell Viability Test

The Haemocytometer and coverslip were thoroughly washed and dried. 100µL of cell suspension was taken and 100µL of Trypan blue was added and mixed. The sample was drawn in Pasteur pipette and gently placed on the counting chamber. The chamber was then focused under a light microscope in low power for observing the cells. The live cells were counted in 1mm square and

cells per ml were calculated.

### DNA Fragmentation

Preparation of agarose gel with 1X TAE buffer and stained with 2µl of ethidium bromide. The 1% of agarose depends upon the molecule to be separated. Samples loaded with loading dye (2µl of loading dye is used). Electrophoresis of DNA fragments at 50 volts. 38 Visualization of DNA fragments in the UV trans-illuminator.

## Results & Discussion

This study was aimed to analyze the anticancer activity of *Tamarindus indica* L. and *Cassia auriculata* L. against breast cancer cell line. The qualitative phytochemical analysis has shown that the extract is positive for saponins, flavonoids, quinones, cardiac glycosides, terpenoids, phenol, coumarins and same extract is negative for carbohydrate, tannins, alkaloids, glycosides, steroids and phytosteroids, phlobatannins and anthraquinones. The DPPH assay test was found to be positive for each extract which can be attributed to the presence of phenols and flavonoids as shown by the Phytochemical screening test. The comparative study

### Sequential Extraction:

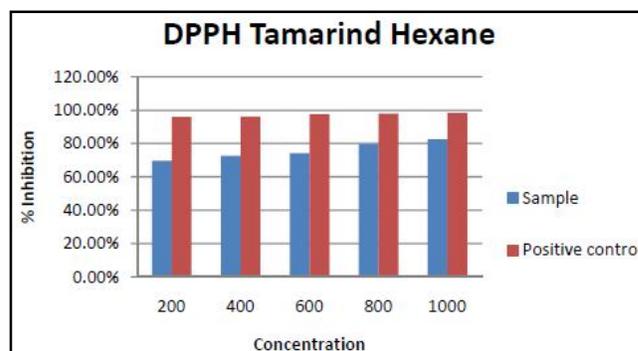
**Table 1:** Results for sequential extraction of percentage yield of plants.

Sl.no	Plant Name	Plant Sequential Extraction		
		Solvent	Yield	
			Initial	Final
1.	Tamarindus indica	Hexane	1.006g	1.053g
		Ethyl acetate	0.791g	0.831g
		Ethanol	1.175g	1.218g
2.	Cassia auriculata	Hexane	1.030g	1.214g
		Ethyl acetate	0.673g	2.540g
		Ethanol	1.190g	3.984g

### Phytochemical analysis:



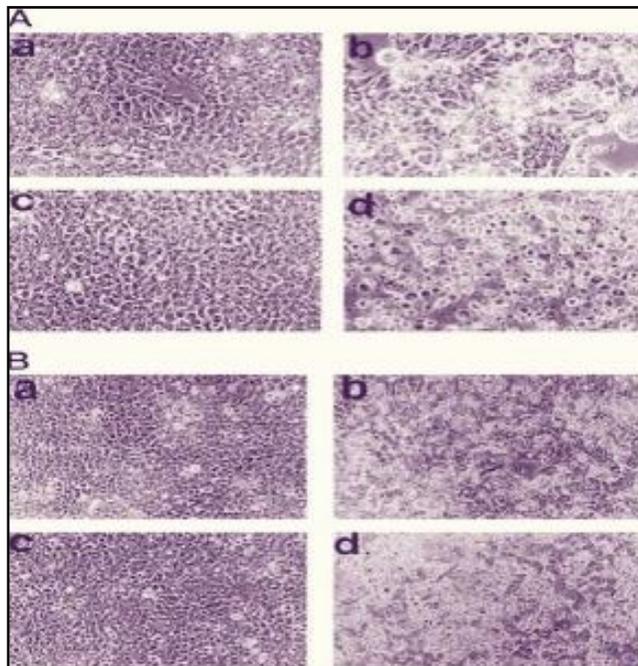
**Fig. 1:** Qualitative test *Cassia auriculata*.



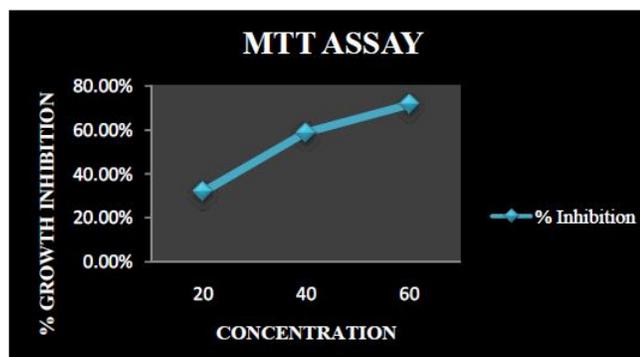
**Fig. 2:** DPPH ASSAY *Tamarindus indica* L. Hexane.

**DPPH Assay:****Table 2:** DPPH ASSAY *Tamarindus indica* L.

Conc. In µg	% Inhibition (C-S)/C*100	% Inhibition (C-P.C)/C*100
200	69.60%	95.90%
400	72.68%	96.14%
600	74.34%	97.46%
800	79.70%	97.85%
1000	82.48%	98.34%

**Invitro Anticancer Activity (MTT Assay):**

**Fig. 3:** MTT assay of MCF 7 cell line. A.pulp 20 µg/ml, c. *in vivo* pulp 40 µg/ml, d. *in vivo* pulp 60 µg/ml) B.control, b. *in vivo* leaf 20 µg/ml, c. *in vivo* leaf 40 µg/ml, d. *in vivo* leaf 60 µg/ml)56 *Tamarindus indica* n conc. for 96 hr in a 96 well plate. Control cells received subjected to MTT within 1 hr for % inhibition on Y axis and Concentration *Tamarindus indica*.



**Fig. 4:** MCF7 Cells were treated with hydro alcoholics of dissolved in DMSO at 20,40 and 60 the solvent used to dissolve the extract. Cells were Response of MCF7 Cell to *Tamarindus indica* on X-axis.

showed the higher antioxidant activity of ethanol and ethyl acetate extract than that of other extract. MTT assay is a universally accepted *in vitro* method for screening the drugs having anticancer activity. The first antitumor drugs from plants with an application in cancer chemotherapy were developed five decades ago. A great achievement in this respect is the elaboration of drugs such as: vinblastine and vincristine (*Catharanthus roseus*), paclitaxel (*Taxus brevifolia*), silvestrol (*Aglaia foveolata*), eliptinium (*Bleekeria vitensis*) (Cragg & Newman, 2005), chrysin (*Passiflora incarnate*), artemisinin (*Artemisia annua*) (Newman & Cragg, 2007) and others.

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