Invitro Anticancer Activity Of Curcuma Amada Against Human Breast Cancer Cell Line Mcf-7

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Abstract

Use of naturally occurring herbs and spices for inhibiting the growth of cancer cells are gaining considerable attention from scientific community due to its low-cost, non-toxicity and high efficacy. Cellular extract of these herbs and spices contains different phytochemicals and bioactive compounds which have shown remarkable inhibition ability for various kinds of cancer cell lines. Herein, we have explored the extract of *Curcuma amada*Roxb in different organic solvents (hexane, chloroform and methanol) for evaluating their cytotoxicity against human breast cancer cell lines (MCF-7) which belongs to family Zingiberaceae used in conventional system of medicine. This important spice is generally used in the preparation of different sauces, pickles, salads and jams. *Curcuma amada* is well known for its anti-inflammatory, antioxidant, and antitumor properties. The main aim of the present work is to investigate the in vitro anticancer activities of the hexane, chloroform and methanolic extracts of *Curcuma amada* against the human breast cancer cell lines [MCF-7]. The average values of (Hexane, Chloroform and Methanolic concerntrations) *Curcuma amada* on the mortality of MCF-7 cells revealed that the Chloroform was more potent than (10:62.9, 20:32.1, 40:3.0, 80:31.5) than Hexane and Methanolic concerntrations. The toxicity of different concentrations of *Curcuma amada* was also investigated against MCF7 cell lines (Hexane, Chloroform and Methanolic concerntrations) which corroborated the potential results. In the present study, Methanol treatment on cell growth of drug-sensitive and Adriamycin-resistant MCF-7 cells where, maximum inhibition was observed.The plant extractinvestigated in this study have significant anticancer activity against the breast cancer cell lines tested. Further investigation is required to isolate and elucidate the structure of the compounds responsible for the observed activity.

Keywords: Curcuma amada, breast cancer, anticancer activity, hexane, chloroform and methanol.
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INTRODUCTION

The increasing cases of cancer among individuals from different sexes, ages, and races is one of the most serious health problems worldwide. Out of total 58 million death of men and women in the world, 13% death was reported due to cancer which turned it into the second leading cause of death. The study conducted by WHO states that the number of cancer patient may crossed 11 million up to 2030. Similarly, in India, the ICMR report has

Among different type of cancer, breast cancer is the most common form of cancer amongst women in India which usually occurs due to the abnormal growth of some breast cells, specifically cells in the milk producing ducts. The breast cancer has become the second largest cause of women death in the world [1]. Therefore, it becomes very important to extend the cancer related studies to get rid of this

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million people by 2025.

disease. There are several therapies have been developed for the treatment of cancer. One of them important one is chemotherapy, but it affects normal cell also. The development of chemical technology has discovered several chemical anticancerous drugs which are toxic and but not economical too. Therefore, there is an urgent need to develop of plant based natural anticancerous compounds for selectively targeting cancerous cells.

Curcuma amada belong to family Zingiberaceae. It is locally known as mango ginger. The local people of Kerala also call it Manghainchior KathumachalorSuraniyika [3-5]. The rhizomes of *Curcuma amada* are pale yellow colored from inside and light-yellow colored from outside with sweet fragrance of unripe mango when crushed [6]. It is also used as antinflamatory agent to relieve pain in poultices [7]. It is being used for centuries as a traditional medicine. It has potential anticancerous and therapeutic properties [8]. The researchers have identified that it suppresses the multiple signaling pathways as well as can inhibit the cell proliferation, invasion, metastasis, and angiogenesis [9, 10]. In Ayurveda and Unani medicinal systems, *Curcuma amada*has been described as an antiinflamatory, antipyretic, diuretic, emollient, expectorant and laxative to cure skin and lungs diseases [11].
The current study has aimed at investigating the anticancerous activity of *Curcuma amada* extract prepared in different solvent.

Materials and Methods

Reagents
The Curcumin and Dimethyl Sulfoxide DMSO used in this study were procured from Sigma-Aldrich. The stock solution Curcumin of 10 mM concentration was prepared with DMSO and stored at -20°C.

Cell Line

The cell lines such as MCF- 7, T-47D, SKBR3, and BT-20, belonged to breast tumor which were procured from the ATCC (Rockville, MD). The MCF-7 cells and BT-20 cells were selected for resistance to adriamycin (MCF- 7 ADR) and tumor necrosis factor (BT-201NF) respectively. Further, these cells were tested against Mycoplasma contamination by using DNA-based assay kit which was procured from Gen-Probe (San Diego, CA).

Cell culture

All breast tumor cell lines were routinely grown in RPMI 1640 medium which were supplemented with HEPES buffer of 10 mM, glutamine of 2 mM, 50 ug/Ml of gentamicin and 10% FCS. These cell lines were kept in a humidified incubator under 5% CO_2 in air and passed twice a week for maintaining exponential growth.

MTT assay

For the determination of number of viable cells which remained after the treatment, the modified tetrazolium salt (MTT) assay was used. Briefly, the cells were incubated at 37°C for 72 h in 5 x 103 wells with sample volume of 2 mL. Further, 0.025 mL of MTT solution (5mg/mL in PBS) was added carefully in each well after extracting 0.1 mL of cell medium. It was followed by the addition of 0.1 mL of the extraction buffer (20% sodium dodecyl sulfate, 50% dimethyl formamide) after incubating at 37°C for 2 h. Thereafter, the optical density was measured at 570 nm using 96 well multiscannerautoreader (Dynatech MR 5000) using extraction buffer as blank. The cell viability was expressed as a percentage using the following equation:

$$\text{Cytotoxicity\%} = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100$$

RESULTS AND DISCUSSION

The curcumin has been an active ingredient in the diet and herbal remedy in several Asian Countries [12]. The research carried out revealed that it has shown potential anticancerous properties in different types of cancerous cells including the tumors of mammary glands [13, 14]. It has become a great interest to find out an effective and novel anticancerous and antitumor phytochemicals which are less toxic to human body. The antiproliferative effects were observed against hormone-independent and -dependent and Adriamycin-sensitive and -resistant breast tumor cells. For investigating the cancer biology, there have been an accessible and easily usable set of models of human cancer cell lines in the previous decades [15]. *Curcuma*

amada species have choosen for investigating this very potential property on the cancer cell lines [16].

The cell lines obtained from the tumor are very easy to us which permits its investigation in a simple and controlled environment [17]. The growth rate of the cells were determined by MTT proliferation assay. The graph plotted between the formazan generated and the number of growth rate of the cells showed a linear relationship and time dependent growth of MCF-7 cells [18]. Therefore, the present study revealed the efficiency of *A. calamus* towards the cytotoxicity against MCF-7 cells which suggested its use for breast cancer.We extended our initial observation regarding the growth inhibitory effect of curcumin to the Adriamycin- resistant breast tumor cell line MCF- 7 ADR. This drug-resistant subclone of MCF- 7 cells was selected by continuous culture using increasing concentrations of Adriamycin over a period of time. The establishment and characteristics of this cell line have been described elsewhere.MCF- 7 ADR cells exhibit a 100- to 120-fold increase in resistance to Adriamycin and express high levels of p-glycoprotein and TGase.24 Unlike their differential sensitivity towards Adriamycin, both the resistant and sensitive MCF- 7 cells showed almost equal susceptibility to curcumin-induced growth inhibition.

The cytotoxic results of different extracts of *C. amada* on MFC-7 cell lines for drug concentration is given in Table 2. The inhibition of 50% growth of MCF-7 cell lines is represented by GI_{50} which was calculated using the equation [(Ti-Tz)/(C-Tz)] x 100 = 50. The concentration of drug resulted from 50% reduction with total increase in protein is represented by TGI which showed the concentration of drug resulted in the inhibition of total growth. The TGI was calculated from Ti = Tz. The concentration of drug at which 50% of the measured protein got reduced was represented by LC50. It is calculated by using the equation [(Ti-Tz)/Tz] x 100 = -50. Here the known drug used were Adriamycin (Doxorubicin). The GI_{50} value ≤ 20µg/mL was selected to show the activity. The activities were marked test values highlighted with Yellow color under GI_{50} column.

The results obtained revealed that the extracts prepared in various solvents (Hexane, Chloroform, Methanol) showed cytotoxicity against MCF-7 cell lines in MTT assay. The LC50 value was calculated as 10.0µg / mL which was responsible for 50% cell death.

The average values of cytotoxic effect of extracts prepared in different solvents are given in.

Table 2. The results clearly revealed that the average values of extracts prepared in Chloroform was more than the

	Human Breast Cancer Cell Line MCF7															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
Ca Hex	35.3	-10.7	-31.1	-37.0	4.2	-22.0	-40.1	-50.1	4.6	-18.3	-46.5	-48.2	14.7	-17.0	-39.2	-45.1
Ca chl	1.6	-43.4	-56.4	-62.1	-9.4	-34.1	-49.6	-50.3	-17.7	-18.2	-31.4	-45.3	-8.5	-31.9	-45.8	-52.5
Ca met	62.9	6.0	-46.0	-62.7	61.6	7.8	-33.0	-54.6	44.2	27.3	6.5	-32.6	56.2	13.7	-24.2	-50.0

extracts prepared in Hexane and Methanol. The graph plotted between different concentrations of extracts of *C. amada* in Hexane, Chloroform and Methanol represented different cytotoxic effect on MFC-7 cell lines (Figure 1). In the present study, Methanol treatment on cell growth of drug-sensitive and adriamycin-resistant MCF-7 cells where, maximum inhibition was observed. The present study is similar to the study of who showed that the hexane, chloroform, and methanol extracts of mango ginger showed the higher toxicity towards cancer cells [19].

Table 1. Average values of (Hexane, Chloroform and Methanolic concerntrations) of *Curcuma amada*

	Drug concentrations (µg/ml) calculated from graph		
MCF7	LC50	TGI	GI50
Ca Hex	75.9	8.2	<10
Ca chl	<10	<10	<10
Ca met	<10	<10	<10

Table 2. MCF-7 cell lines treated with hexane,

chloroform and methanolic concerntrations of *Curcuma amada* and drug concerntrationscalculated from graph. LC-indicates Concerntration of the drug,TGI-indicates Total Growth Inhibition,GI-indicates Growth Inhibition. Activities were marked in Yellow highlighted test values under GI50 column.

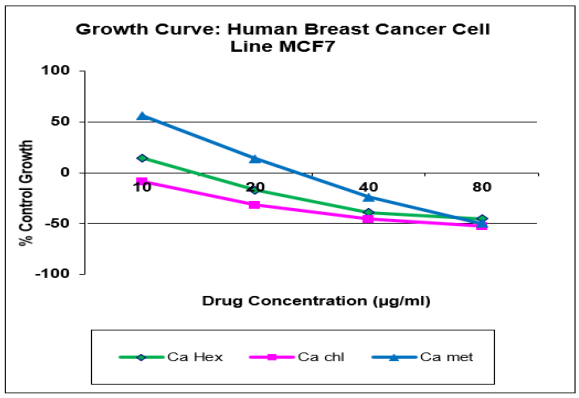

Figure 1 Effect of *Curcuma amada* treatment on cell growth of drug-sensitive and Adriamycin-resistant MCF-7 cells.

CONCLUSION

The breast cancer has been spreading day by day in Indian women. The different therapies developed till date like chemotherapy, radiation therapy has shown negative impact on the normal cells too. One of the major disadvantage of such therapy is that these are effective for only one type of cancerous cell. Therefore, the need of effective and potential anticancerous agent which can overcome on the mentioned disadvantages is of great interest. Various phytochemical have shown their effect on cancerous cell with no side-effects. The chief active constituent of *C. amada* extracts have great therapeutic properties which can cure several health related issues including jaundice, hemorrhage, menstrual difficulties, flatulence, hematuria, etc. The results showed that the methanolic extract of C. amada has potent anticancerous activity against MCF-7 cell lines. Therefore, *C. amada* can be used as an anticancerous agent. Nevertheless, further research and development are very necessary to investigate its use and mode of action.

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