INVITRO ANTICANCER ACTIVITY OF CURCUMA LONGA AGAINST HUMAN BREAST CANCER CELL LINE MCF-7

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ABSTRACT
Turmeric (Curcuma longa) commonly known as Halad in Marathi and Haldi in Hindi is an Indian spice and medicinal plant belonging to Zingiberaceae family and is expensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. Breast cancer is a significant cause of death among women worldwide. The purpose of this study is to test in vitro anticancer activities of the hexane, chloroform and methanolic extracts of Curcuma longa against the human breast cancer cell lines [MCF-7]. The Average values of (Hexane, Chloroform and Methanolic concerntrations) of Curcuma longa were shown in (Table 1), Out of which Chloroform Average values was found to be more 10:62.9, 20:32.1, 40:3.0,80:31.5) than Hexane and Methanolic concerntrations.Agraph was plotted between different concentrations of Curcuma longa (Hexane, Chloroform and Methanolic concerntrations) had different cytotoxicity effects on MCF7 cell line (Figure 1). The plant extract investigated 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 longa, breast cancer, anticancer activity, hexane, chloroform and methanolic.

INTRODUCTION
Cancer is one of the most serious health problems worldwide, affecting individuals from different sexes, ages, and races. In 2005, cancer was the second leading cause of death among
both men and women and accounted for 13% of the total 58 million deaths worldwide. In 2006, about 10.9 million new cancer cases are expected to be diagnosed worldwide and more than 7.8 million cancer patients may die. Cancer is also a problem of economical dimensions with a very high level of expenses associated to it. For example the National Institute of Health, USA estimates that an overall of $209.9 billion were invested worldwide in 2005, for the sake of cancer research and management.

It is known that breast cancer is the most common cancer for women worldwide, and accounts for approximately 25% of all female malignancies with a higher prevalence in developed countries. Breast cancer is the second leading cause of cancer-related death among females in the world.[1] The discovery of novel natural compounds with low toxicity and high selectivity for killing cancer cells is an important area in cancer research.[2] To date, chemotherapy has been the most frequently used treatment for breast cancer and other cancers. However, this method of treatment also destroys some normal cells as well. Curcumin or diferuloylmethane is the major yellow pigment extracted from turmeric (Curcuma longa) and is commonly used as a flavoring agent in food.[3]

Moreover, extensive research has shown that curcumin possesses antiproliferative and anti-carcinogenic properties in a wide variety of cell lines and animals.[4] Curcuma longa is a medicinal plant which its constituents, especially curcumin, has diverse anti-cancer properties including anti-telomerase action.[5,6] The present study aimed to evaluate the possible cytotoxic activity of the rhizomes of Curcuma longa against human breast cancer cell line.

MATERIALS AND METHODS
Reagents
Curcumin was purchased from Sigma-Aldrich Corporation and was prepared with Dimethyl Sulfoxide (DMSO) at a concentration of 10 mM, stored as small aliquots at -20°C, and thawed and diluted as needed in cell culture medium.

Cell Line
The human breast tumor cell lines BT-20, T-47D, SKBR3 and MCF-7 were obtained from the ATCC (Rockville, MD). The MCF-7 cells were selected for resistance to adriamycin (MCF-7 ADR) and BT-20 cells were selected for resistance to tumor necrosis factor (BT-201NF). Cells were tested for Mycoplasma contamination using either the DNA-based assay kit purchased from Gen-Probe (San Diego, CA) or the Hoechst stain.
Cell culture
All breast tumor cell lines were routinely grown in RPMI 1640 medium supplemented with 10 mM HEPES buffer, 2 mM glutamine, 50 μg/ml gentamicin and 10% FCS. The cells were cultured in a humidifled incubator in 5% CO2 in air and were maintained in continuous exponential growth by twice a week passage.

MTT assay
The number of viable cells remaining after appropriate treatment was determined by using the modified tetrazolium salt (MTT) assay as described. Briefly, 5 X 10^3 cells/well were incubated in the presence or absence of the indicated test sample in a final volume of 0.2 ml for 72 h at 37°C. Thereafter, 0.1 ml of cell medium was removed and 0.025 ml of MTT solution (5 mg/ml in PBS) was added to each well. After 2 h incubation at 37°C, 0.1 ml of the extraction buffer (20% sodium dodecyl sulfate, 50% dimethyl formamide) was added. After an overnight incubation at 37°C, the optical densities at 570 nm were measured using a 96-well multiscanner autoreader (Dynatech MR 5000), with the extraction buffer serving as a 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
For centuries, curcumin has been consumed in the diet and used as a herbal medicine in several Far Eastern Countries.[7] Curcumin has cancer chemopreventive properties in a variety of animal models of chemical carcinogenesis, including those resulting in tumors of the mammary gland.[8,9] The search for new chemopreventive and antitumor agents that are more effective and less toxic has kindled great interest in phytochemicals. The antiproliferative effects were observed against hormone-independent and -dependent and adriamycin-sensitive and -resistant breast tumor cells. In the last few decades, human cancer cell lines have aggregated an accessible, easily usable set of biological models to examine cancer biology.[10]

The utility of cell lines acquired from tumor allows the investigation of tumor cells in a simplified and controlled environment.[11] MTT proliferation assay was carried out to determine the growth rate of cells. A linear relationship between the formazan generated and the number of viable cells was demonstrated, together with time-dependent growth
characteristics for MCF-7 cells by.\textsuperscript{[12]} Hence present study shows the efficacy of A.calamus for the cytotoxicity towards MCF-7 cells thus suggesting protection against 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 in the presence of 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.\textsuperscript{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 results for cell growth inhibition by the Hexane, Chloroform and methanolic extracts of \textit{Curcuma longa} on MCF-7 cell lines for Drug concentrations is shown in Table 2. Chloroform extract of \textit{Curcuma longa} was found to be cytotoxic towards human MCF-7 in MTT assay and the concentration required for 50% cell death was found to be 11.0\textmu g / ml. The Average values of (Hexane, Chloroform and Methanolic concentrations) of \textit{Curcuma longa} were shown in (Table 1), Out of which Chloroform Average values was found to be more 10:62.9, 20:32.1, 40:3.0,80:31.5 than Hexane and Methanolic concentrations. A graph was plotted between different concentrations of \textit{Curcuma longa} (Hexane, Chloroform and Methanolic concentrations) had different cytotoxicity effects on MCF7 cell line (Figure 1). In the present study, Choloform treatment on cell growth of drug-sensitive and adriamycin-resistant MCF-7 cells where, maximum inhibition was observed.

\textbf{Table 1: Average values of (Hexane, Chloroform and Methanolic concentrations) of \textit{Curcuma longa}.}

<table>
<thead>
<tr>
<th>Drug Concentrations (\textmu g/ml)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Average Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Cl Hex</td>
<td>10.2</td>
<td>2.6</td>
<td>12.0</td>
<td>-</td>
</tr>
<tr>
<td>Cl Chl</td>
<td>76.0</td>
<td>66.2</td>
<td>29.4</td>
<td>-</td>
</tr>
<tr>
<td>Cl met</td>
<td>17.0</td>
<td>16.1</td>
<td>10.0</td>
<td>0.2</td>
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</tbody>
</table>
Table 2: MCF-7 cell lines treated with hexane, chloroform and methanolic concentrations of *Curcuma longa* and drug concentrations calculated from graph.

<table>
<thead>
<tr>
<th></th>
<th>MCF7</th>
<th>LC50</th>
<th>TGI</th>
<th>G150</th>
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<tbody>
<tr>
<td>Cl Hex</td>
<td>&gt;80</td>
<td>49.5</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Cl Chl</td>
<td>&gt;80</td>
<td>50.7</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Cl met</td>
<td>&gt;80</td>
<td>52.3</td>
<td>&lt;10</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Effect of *Curcuma longa* treatment on cell growth of drug-sensitive and adriamycin-resistant MCF-7 cells.

**CONCLUSION**

Turmeric is safe and non-toxic for most patients. It has been shown to have diverse biological effects in humans and animals. The evidence suggests that it can suppress tumorigenesis, tumor promotion, and metastasis and, therefore, has enormous potential as an anticancer agent. Due to its potential as an antitumor remedy, use of turmeric for the treatment and prevention of cancer should be considered, except during chemotherapy.

The study concludes that *Curcuma* rhizome may be a promising natural source for active compounds against malignant melanoma. The presented results has been shown that *Curcuma longa* (Chloroform) extract exhibited anti-cancer activity and acts as a potent growth suppressive agent against Human breast cancer MCF-7.
REFERENCES


