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RESEARCH ARTICLE

Invitro Anticancer Activity of *Hedychium coronarium* Against Human Breast Cancer Cell Line MCF-7

Prasanthi Donipati^{1*}, Dr. S. Hara Sreeramulu²

1. Research Scholar, Dept. of Biotechnology, Dr.V.S.Krishna Govt. College

2. Professor & Head of the Dept. of Biotechnology, Dr.V.S.Krishna Govt. College, Visakhapatnam, A.P, India-530 013.

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*Corresponding Author

Prasanthi Donipati

Abstract

Hedychium coronarium Koen. (Family Zingiberaceae), popularly named butterfly ginger, is widely available in tropical and subtropical regions. The purpose of this study was to test in vitro anticancer activities of the hexane, chloroform and methanolic extracts of *Hedychium coronarium* against the human breast cancer cell lines [MCF-7]. The Average values of (Hexane, Chloroform and Methanolic concentrations) of *Hedychium coronarium* were shown in (Table 1), Out of which Methanol Average values was found to be more 10:100, 20:100, 40:100,80:94.2) than Hexane and Methanolic concentrations. A graph was plotted between different concentrations of *Hedychium coronarium* (Hexane, Chloroform and Methanolic concentrations) had different cytotoxicity effects on MCF7 cell line (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 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.

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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. *Curcuma* Linn. is a large genus belonging to the family Zingiberaceae. It comprises about 70 species of rhizomatous herbs distributed mostly in Southeast Asia as wild and cultivated plants

^[3]The Zingiberaceae plant *Hedychium coronarium* Koen., which has many common names including butterfly ginger, butterfly lily, cinnamon jasmine, garland flower and ginger lily is widely available in tropical and subtropical regions, such as Japan, India, Brazil, South China, Southeast Asian countries and so on. The rhizome of *H. coronarium* ("Tuqianghuo" in Chinese) has been used for the treatment of headache, diabetes, contusion inflammation and sharp pain due to rheumatism in Chinese traditional medicine, while it is also used as a febrifuge, tonic, excitant and anti-rheumatic in the Ayurvedic system of traditional Indian medicine ^[4]. It has been reported that its rhizomes are used for the treatment of diabetes, tonsillitis, infected nostrils, tumor and fever ^[5,6]. The present study aimed to evaluate the possible cytotoxic activity of the rhizomes of *Hedychium coronarium* 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 humidified 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 (MTI) assay as described. Briefly, 5 X 10³ 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 ^[12]. Hence present study shows the efficacy of *Hedychium coronarium* 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.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 *Hedychium coronarium* on MCF-7 cell lines for Drug concentrations is shown in Table 2., GI50 showed Growth inhibition of 50 % (GI50) calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, drug concentration resulting in a 50% reduction in the net protein increase, TGI showed Drug concentration resulting in total growth inhibition (TGI) will calculated from $Ti = Tz$, LC50 showed Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$ and Adriamycin (Doxorubicin) was used as the known drug. GI50 value $\leq 20\mu\text{g/ml}$ is considered to demonstrate activity. Activities were marked in Yellow highlighted test values under GI50 column.

Chloroform extract of *Hedychium coronarium* was found to be cytotoxic towards human MCF- 7 in MTT assay and the concentration required for 50% cell death was found to be $10.0\mu\text{g / ml}$ as shown in (Table 2). The Average values of (Hexane, Chloroform and Methanolic concentrations) of *Hedychium coronarium* were shown in (Table 1), Out of which Methanol Average values was found to be more 10:100, 20:100, 40:100,80:94.2) than Hexane and Methanolic concentrations. A graph was plotted between different concentrations of *Hedychium coronarium* (Hexane, Chloroform and Methanolic concentrations) had different cytotoxicity effects on MCF7 cell line (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.

	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
Hc Hex	-29.0	-55.7	-58.5	- 71.0	-43.7	-45.1	-47.5	- 60.9	-48.6	-49.0	-50.6	-63.6	-40.4	-49.9	-52.2	- 65.1
Hc chl	15.0	-12.7	-56.8	- 62.7	12.0	-14.3	-42.8	- 53.9	10.2	-14.3	-53.9	-57.1	12.4	-13.8	-51.2	- 57.9
Hc Met	100.0	100.0	100.0	95.6	100.0	100.0	100.0	86.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	94.2
ADR	100.0	27.8	-54.8	- 61.7	92.6	74.2	-13.9	- 60.7	95.2	90.7	0.0	-45.4	95.9	64.2	-22.9	- 55.9

Table 1. Average values of (Hexane, Chloroform and Methanolic concentrations) of *Hedychium coronarium*.

Drug concentrations ($\mu\text{g/ml}$) calculated from graph			
MCF7	LC50	TGI	GI50
Hc Hex	<10	<10	<10
Hc chl	<10	<10	<10
Hc Met	>80	>80	>80
ADR	70.13	46.94	23.74

Table 2. MCF-7 cell lines treated with hexane, chloroform and methanolic concentrations of *Hedychium coronarium* and drug concentrations calculated from graph. LC-indicates Concentration of the drug, TGI-indicates Total Growth Inhibition,GI-indicates Growth Inhibition. Activities were marked in Yellow highlighted test values under GI50 column.

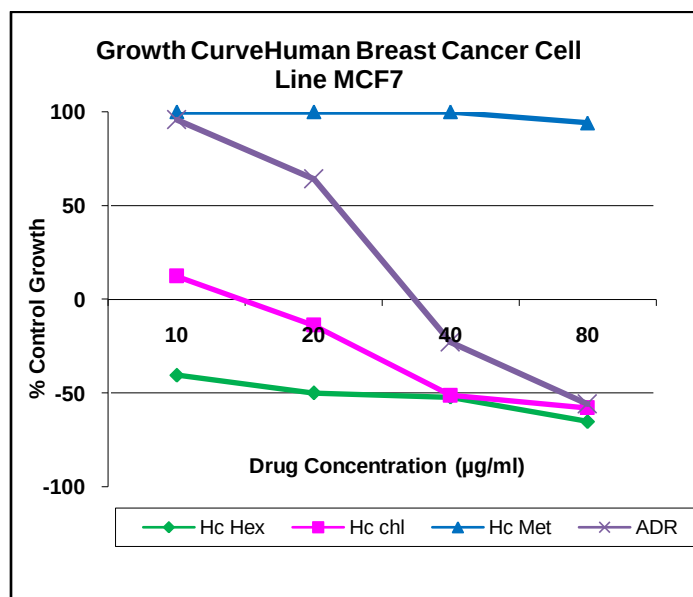


Figure 1 Effect of *Hedychium coronarium* treatment on cell growth of drug-sensitive and adriamycin-resistant MCF-7 cells.

CONCLUSION

The incidence of breast cancer has been increasing among Indian women. The current therapies such as chemotherapy, radiation therapy, hormonal therapy and surgery cause severe side effects. The disadvantages of these therapies also include their effectiveness towards only one type of cancer. Hence, natural products from plant sources have been used in the treatment of cancer, which reduces the risk and potential side effects associated with the other forms of therapies. The *Hedychium coronarium* rhizome has anti-cancerous, antioxidant, anti-hypertensive, diuretic, leishmanicidal, anti-malarial activities and were also used in irregular menstruation, piles bleeding and stone in urinary tract. Cancer chemoprevention activity is also reported recently of labdane diterpenes from rhizomes of *Hedychium coronarium*. The medicinal value of this plant in the treatment of a large number of human ailments was mentioned in Ayurveda, Charaka Samhita and Sushruta Samhita [4]. The presented results have shown that *Hedychium coronarium* (Methanol) extract exhibited anti-cancer activity and acts as a potent growth suppressive agent against Human breast cancer MCF-7.

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