ORIGINAL RESEARCH PAPER

Pharmaceutical Science

IN VITRO ANTICANCER ACTIVITY OF LEAF EXTRACT OF COUROUPITA GUIANENSIS AGAINST COLON CANCER CELL LINE

KEY WORDS: Couroupita guianensis, Lecythidaceae, Colon cancer cell line, MTT assay.

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ABSTRACT

Objective: This study aims in dealing with the in vitro anticancer activity of leaves of Couroupita guianensis (family: Lecythidaceae) against Colon Cancer cell line(Colo205). Materials and Methods: The in vitro anticancer activity of methanolic leaf extract of Couroupita guianensis was studied against Colon cancer cell line(Colo205) using MTT Assay. Results: The methanolic extract of leaves (CgL)of Couroupita guianensis showed good percentage of inhibition against Colo205 cell line as compared to standard drug. Conclusion: The study provide information which may generate interest among researchers.

INTRODUCTION

Colon cancer is a type of cancer in which the body cells that lines the colon grows abnormally. People across the world are less aware of this disease. Cases of colon cancer are increasing day by day. Studies shows that there is 2% increase in cases every year. Colon cancer mainly affect older adults. It usually begins as polyps that form on the inside of the colon and some of these polyps can become colon cancers. Sometimes colon cancer is also called as Colorectal cancer, a term which combines colon cancer and rectal cancer.

For the treatment of this chronic disease traditional medicinal plant have been used. Example of one such traditional medicinal plant is Couroupita guianensis belonging to the family Lecythidaceae. It is also known as Cannonball tree.. It originates in Central and South American tropical forests. It has many potential medicinal uses. One of the active constituent of Couroupita guianensis is Isatin an endogenous compound having cytotoxic activity against human cancer cell line. The leaves of Couroupita guianensis is mainly used to treat skin diseases.

Figure no:1 Leaves of Couroupita guianensis

MATERIALS AND METHODS

Plant Collection And Extraction

The *Couroupita guianensis* leaves were gathered at St. Thomas College in Kozhencherry, Kerala. The leaves were equally pulverised and kept after being driedusing the shade drying process. 350g of coarsely ground Couroupita guianensis leaves were treated to separate and subsequent macerations with 70 percent methanol and distilled water. The MTT Assay was carried out using the leaf extract.

In-vitro Anticancer Activity

The MTT assay measures cell metabolic activity via colorimetry. It is predicated on the capacity of cellular oxidoreductase enzymes to convert the purple formazan, an insoluble form of the tetrazolium dye MTT, to NADPH-dependent levels. Utilizing cellular metabolic activity as a gauge of cell viability, proliferation, and cytotoxicity is the MTT test.

This colorimetric assay relies on the transformation of purple formazan crystals into a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)2,5diphenyltetrazolium bromide, or MTT) by metabolically active cells. The MTT is converted to formazan by the NAD(P)H-dependent oxidoreductase enzymes found in the live cells. A solubilization solution is used to dissolve the insoluble formazan crystals, and a multi-well system is used to measure the absorbance at 500–600 nanometers.

In Vitro Anticancer Activity Of Leaf Extract Of Couroupita Guianensis

Cell line: Colo205 (Colon Cancer cell line)

Media: RPMI 1640 with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No -10270106 Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062

Experimental procedure: MTT Assay

The cells were incubated for 24 hours at 5 percent CO_2 and 37°C at a concentration of 1× 104 cells/ml. 100 µl of Couroupitaguianensis leaves sample (CgL) and 70 µl of cells in culture medium were added to microplates at a concentration of 10, 40, and 100 µg/ml, respectively (96 wells and tissue culture grade). Cell line and DMSO (0.2 percent in PBS) were incubated in the Control wells. Triplicates of each specimen were incubated. The proportion of living cells after culture and control cell survival were calculated using controls that were kept in place. The cell cultures were then kept in a CO_2 incubator for 24 hours at 5% CO_2 and 37°C (Thermo scientific Bb150).

After incubation, the media was completely withdrawn, 20 µl

of MTT reagent (5 mg/min PBS) was added, and the cells were then incubated for 4 hours at 37 °C in a CO_2 incubator. The wells were then examined under a microscope to check for the production of formazan crystals. Only live cells reduced the yellow MTT to a dark-colored formazan. After fully removing the medium 200 µl of DMSO were added and incubated at 37°C for 10 minutes (wrapped with aluminium foil). The absorbance of each sample was determined using a microplate reader (Benesphera E21) at a wavelength of 550 nm to evaluate the triplicate samples.

Figure 2: CO_2 incubato

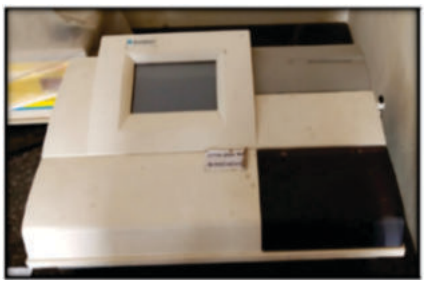

Figure 3: Elisa plate readerRESULTS:

RESULTS:

In-vitro Anticancer Activity Of Leaf Extract Of Couroupita Guianensis

Table 1: Effects of compound against Colo205 (Colon cancer cell line) by MTT assay

Sr. no	Sample	Concentration(µg/m l)	OD	% inhibition	IC 50 (µg/ml))
1	Control		2.414		
2	Std. 5 FU	10	0.669	72.28	45.35
		40	0.589	75.60	
		100	0.423	82.47	
3	Cg L	10	1.011	58.11	46.93
		40	0.931	61.43	
		100	0.719	70.21	

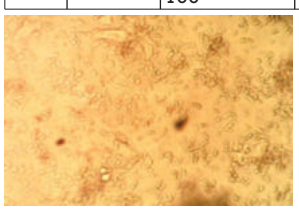

Figure 4(a) Control (0.1% DMSO treated)

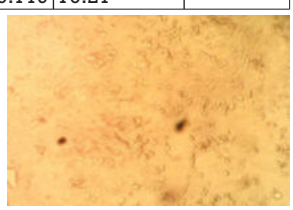

Figure 4(b) Standard (5 FU)

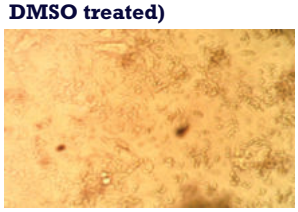

Figure 4(c) Treatment CgL (40µg/ml)

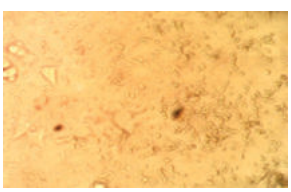

Figure 4(d) Treatment CgL (100µg/ml)

Figure 4: In-vitro anticancer activity of leaf extract of Couroupita guianensis

RESULT:

The antiproliferation impact to be evaluated when resea rching anticancer agents, hence anticancer activity of leaf extract of *Couroupita guianensis*(CgL) was evaluated on Colo205 (a colon cancer cell line) at various concentrations. The CgL extract with IC 50 value of 46.93µg/ml had highest toxicity on Colo205 when compared to standard (5 FU).

DISCUSSION

By using the MTT assay, the in vitro anticancer activity of methanolic extract of leaves was examined against the colon cancer cell line (Colo205). We found that, at a concentration of 46.93µg/ml, the methanolic extract of leaves of Couroupita guianensis demonstrated good percent inhibition of the Colo205 cell line when compared to standard drug.

The leaf extract(CgL) with IC 50 value of 46.93µg/ml had highly prevent the cell proliferation in the dose dependent manner when compared to the standard drug. So it might be utilised as a chemotherapeutic drug to treat cancer

CONCLUSION

Cancer continues to be the top cause of death in the globe. Early diagnosis and treatment can cure it, although there are many difficulties with it. By using the MTT assay, it was discovered that the methanolic extract of leaves of Couroupita guianensis had a good percent inhibition against the Colon cancer cell line(Colo205) at the concentration of 46.93µg/ml as compared to the standard. The current attempt offers details that might generate interest among researchers to explore such natural resources.

The result of the study suggest that the methanolic extract of leaves of Couroupita guianensis has anticancer activity and further investigation can be carried out to develop a potent and safe anticancer drug.

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