Original Research Paper

Volume - 12 | Issue - 08 | August - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pharmacy

IN VITRO ANTICANCER ACTIVITY OF FLOWERS OF COUROUPITA GUIANENSIS AGAINST SKIN CANCER CELL LINE

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ABSTRACT **Objective:** This study aim's in dealing with the in vitro anticancer activity of flowers of Couroupita guianensis (family: Lecythidaceae) against B16F10 (skin cancer cell line). **Materials and Methods:** MTT assay for flowers of Couroupita guianensis against B16F10 (Skin Cancer cell line) is conducted. **Results:** At the Concentration 44.44µg/ml, methanolic flower extract of Couroupita guianensis showed good percent inhibition B16F10(Skin Cancer cell line) as compared to standard drug. **Conclusion:** The present attempt provides information which may generate interest among researchers to explore such natural resources.

KEYWORDS : Couroupita guianensis(CgF), Lecythidaceae, MTT Assay, Skin cancer cell line

INTRODUCTION

Skin cancer is one of the most common type. Every year, about million cases of skin cancer are discovered. Squamous cell carcinoma and basal cell carcinoma which are various type of non-melanoma skin cancers, are not likely to spread and may need little more than minor surgery or topical care. Melanoma, which accounts for about 1 % of all skin cancers but it is the main cause for most skin cancer deaths, may spread (metastasize) through the bloodstream to other body parts and lymphatic system.

Couroupita guianensis (CgF) also known as Cannonball tree (family: Lecythidaceae), Nagalinga pushpam. The native of Couroupita guianensis is tropical forests of Central and South America, and it is cultivated in many other tropical areas throughout the world.

Isatin(1H-indole-2,3-dione) is one of the active constituent in Couroupita guianensis. It is an endogenous compound which is found to have cytotoxic activity against human cancer cell line. Isatin has been isolated from the flower of Couroupita guianensis to study anticancer activity in various cell lines.

Fig 1 :Flower of Couroupita guianensis

MATERIALS AND METHODS

PLANT COLLECTION

The flowers of Couroupita guianensis were collected from St. Thomas College, Kozhencherry, Kerala. The flowers were dried using shade drying method, powdered uniformly and stored. 100g coarsely powdered flowers of Couroupita guianensis was subjected to maceration separately and successively with 70% methanol and distilled water. The flower extract was used to conduct MTT assay.

IN-VITRO ANTICANCER ACTIVITY

Cell proliferation and viability are analyzed using most widely used MTT assay method. The metabolic activity of the cell is measured by MTT assay which is a colorimetric assay. It is based on the capacity of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzymes to reduce the tetrazolium dye MTT to its purple color insoluble formazan.

This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) by metabolically active cells to purple formazan crystals. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan. By using a solubilization solution the insoluble formazan crystals are dissolved and the resulting colored solution is quantified by measuring absorbance at 500-600 nanometers using a multi-well spectrophotometer. The darker the solution, the greater the number of viable, metabolically active cells.

In vitro Anticancer activity of flowers of Couroupita guianensis

Cell line: B16F10 (Skin Cancer cell line)

Media: DMEM 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 at a concentration of 1×10^4cells/ml for at 5% Carbondioxide and 37°C for 24 hours .Cells were seeded in 100 µl culture medium at a concentration (70µl) 10^4cells/well and 100µl sample of flowers of Couroupita guianensis in (10,40,100µg/ml) into micro plates respectively (96 wells and tissue culture grade). The Control wells were incubated with DMSO (0.2% in PBS) and cell line. All specimen were incubated in triplicate. controls were maintained and it was used to determine the control cell survival and the percentage of live cells after culture.Then the cell cultures were incubated for 24 hours at 5% Carbondioxide and 37°C in Carbondioxide incubator (Thermo scientific Bb150).

Figure 2: CO_2 incubator

After incubation, the medium was completely removed and added 20 µl of MTT reagent(5mg/min PBS), after addition of MTT, cells incubated for 4 hours at 37°C in CO2incubator, then observed the wells for formazan crystal formation under microscope. The yellowish MTT was decreased to dark colored formazan by viable cells only. After removing the medium completely. Added 200 µl of DMSO (kept for 10 min) and incubate at 37°C (wrapped with aluminium foil). Triplicate samples were analyzed by measuring the absorbance of each sample

RESULTS

In-vitro Anticancer activity of flowers of Couroupita guianensis(CgF)

Table 1: Effects of compound against B16F10 Cell line (Skin cancer cell line) by MTT assay

Sr. no	Sample	Concentration (µg/ml)	OD	% inhibition	IC 50 (µg/ml))
1	Control		0.914		
2	Std. 5 FU	10	0.551	39.71	30.25
		30	0.431	52.84	
		100	0.409	55.25	
3	CgF	10	0.713	22.10	44.44
		30	0.646	29.32	
		100	0.549	39.93	

Figure 4: In vitro Anticancer activity of flowers of Couroupita guianensis

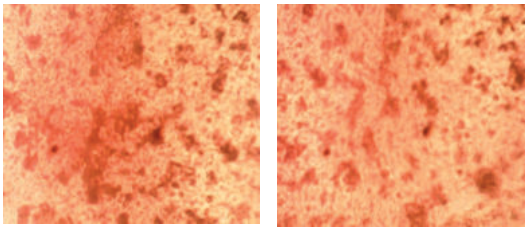

Figure 4(a) Control (0.1% DMSO PBS treated)
Figure 4(b) Standard (5 FU)

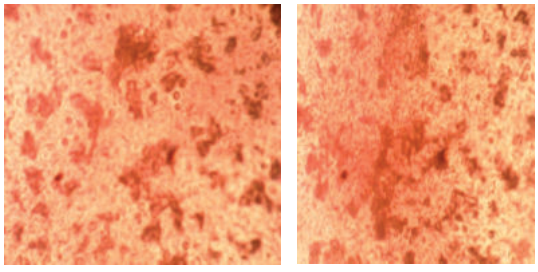

Figure 4(c) Cg F treated (40 µg/ml)
Figure 4(d) Cg F treated (100 µg/ml)

Result:

The antiproliferation effect is the first indication to be assessed when investigating antitumor agents, thus the cell growth inhibitory activity of CgF was assessed on B16F10 Cell line (Skin cancer cell line) at different concentration. A dose dependence decrease in cell viability was observed at an IC 50 value of 44.44 µg/ml.

DISCUSSION

The anticancer activity of methanolic extract of flowers was studied against skin cancer cell line (B16F10) by MTT assay and we observed that at the concentration 44.44µg/ml, methanolic extract of flowers of Couroupita guianensis showed good percent inhibition B16F10 cell line as compared to standard drug.

Cytotoxicity against B16F10 (skin cancer cell line) was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide assay (MTT assay) .The vital compound present in C. guianensis is isatin that shows solid inhibitory activity with IC 50 value of 44.44µg/ml in a dose-dependent manner against standard 5 FU. The flower of Couroupita guianensis consists of isatin compound that has cytotoxicity against human carcinoma cell lines. It has the potential to be used as a chemotherapeutic agent against cancer. Isatin isolated from floral parts exhibited cytotoxicity against various cell line

CONCLUSION

Cancer remains a leading cause of death worldwide, as a result of challenges including increased toxicity and development of resistance to treatment agents. Anticancer activity of methanolic extract of flowers of Couroupita guianensis against cell line B16F10 (Skin cancer cell line) by MTT assay was found to have a good percent inhibition at the concentration of 44.44 µg/ml as compared to the standard. The present attempt provides information which may generate interest among researchers to explore such natural resources. Outcomes of the study suggests that flower extract of Couroupita guianensis is intoxicating chemo preventive and supplemental invivo animal studies are necessitated to demonstrate extract as safe molecules for cancer management.

ACKNOWLGEDMENT

We are thankfull to the management of Nazareth College Of Pharmacy, Othera, Thiruvalla, Kerala for the help and support and Dr. P. Selvam, Aravindh Herbal Labs Pvt.Ltd, Tamil nadu.

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