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

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Keywords:

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ABSTRACT: Objective: This study aim's to deal 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 44.44 µg/ml, the methanolic flower extract of *Couroupita guianensis* showed good percent inhibition of B16F10 (Skin Cancer cell line) as compared to the standard drug. **Conclusion:** The present attempt provides information that may generate researchers' interest in exploring such natural resources.

INTRODUCTION: Skin cancer is one of the most common types. Every year, about a million cases of skin cancer are discovered. Squamous and basal cell carcinoma, which are various types 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 is the main cause of most skin cancer deaths, may spread (metastasize) through the bloodstream to other body parts and the lymphatic system. *Couroupita guianensis* (CgF), also known as Cannonball tree (family: Lecythidaceae), Nagalinga pushpam. The native of *Couroupita guianensis* is in 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 that has cytotoxic activity against human cancer cell lines. Isatin has been isolated from the flower of *Couroupita guianensis* to study anticancer activity in various cell lines.

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FIG. 1: FLOWERS 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* were 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 by the most widely used MTT assay method. The cell's metabolic activity is measured by MTT assay, 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. The insoluble formazan were dissolved by solubilizing solution 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 or 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 1×10^4 cells/ml at 5% CO_2 and 37°C for 24 hours. Cells were seeded in 100 µl culture medium at a concentration (70µl) 10^4 cells/well and 100µl sample of flowers of *Couroupita guianensis* (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 specimens 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% CO_2 and 37°C in CO_2 incubator (Thermo scientific BB150).

FIG. 2: CO_2 INCUBATOR

FIG. 3: ELISA PLATE READER

After incubation, the medium was completely removed and added 20 µl of MTT reagent (5mg/min PBS), after the addition of MTT, cells are incubated for 4 hours at 37°C in CO_2 incubator, then observed the wells for formazan crystal formation under a microscope. The yellowish MTT was decreased to dark-colored formazan by viable cells only. After removing the medium completely, add 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 with a microplate reader (Benesphera E21) at a wavelength of 550 nm.

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		30.25
2	Std. 5 FU	10	0.551	39.71	
		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	

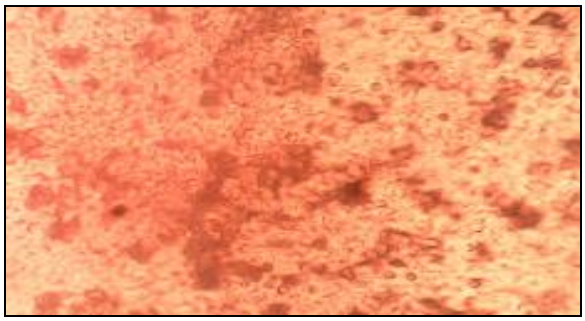

FIG. 4(A): CONTROL (0.1% DMSO PBS TREATED)

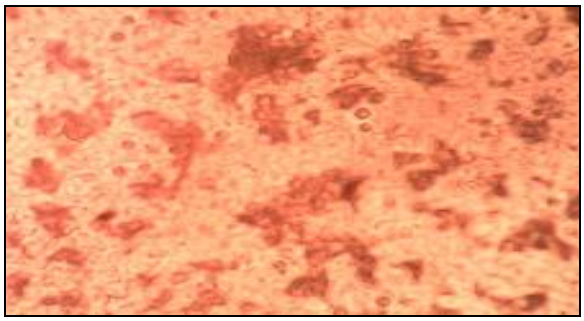

FIG. 4(B): STANDARD (5 FU)

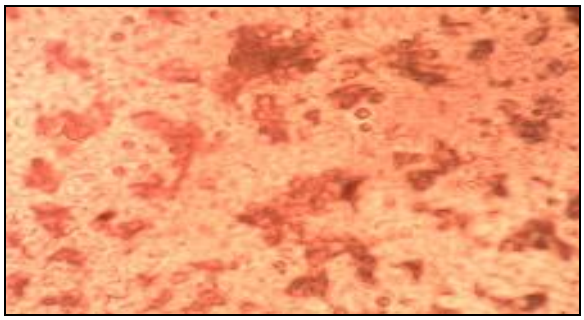

FIG. 4(C): CG F TREATED (4 µg/ml)

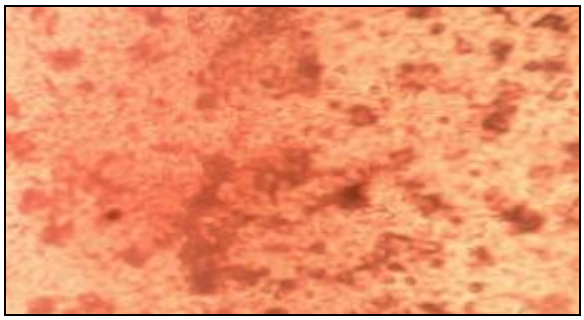

FIG. 4(D) CG F TREATED (100 µg/ml)

FIG. 4: *IN-VITRO* ANTICANCER ACTIVITY OF FLOWERS OF *COUROUPITA GUIANENSIS*

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 concentrations. A dose-dependence decrease in cell viability was observed at an IC 50 value of 44.44µg/ml.

DISCUSSION: The anticancer activity of methanolic extract of flowers was studied against Skin cancer cell line (B16F10) by MTT assay and 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 (5-FU).

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 which shows solid inhibitory activity with IC_{50} value of 44.44µg/ml in a dose-dependent manner aganist standards 5 FU. The flower of *Couroupita guianensis* consists of an 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 in nowadays. A plan for the diagnosis and treatment of cancer is a key component of any overall cancer control plan. 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 that may generate

researchers' interest in exploring such natural resources. Outcomes of the study suggest that the flower extract of *Couroupita guianensis* is intoxicating chemopreventive and supplemental *in-vivo* animal studies are necessary to demonstrate extract as safe molecules for cancer management.

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CONFLICT OF INTEREST: Nil

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