



Received on 01 August 2022; received in revised form, 10 December 2022; accepted, 14 December 2022; published 31 December 2022

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

K. S. Nisana^{*}, Alkka Anna Sabu, Ansa Elezabeth Mathew, Chinchu K. Moncy, B. Gopika, V. Jiju and Elessy Abraham

Nazareth College of Pharmacy, Othara, Thiruvalla - 689546, Kerala, India.

Keywords:

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

Correspondence to Author:

K. S. Nisana

Nazareth College of Pharmacy,
Othara, Thiruvalla - 689546, Kerala,
India.

E-mail: nisanaks7790@gmail.com

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.



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

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.IJP.9(12).202-05
	The article can be accessed online on: www.ijppjournal.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.9(12).202-05	

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.



FIG. 2: CO₂ INCUBATOR

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 CO₂ 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,

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% CO₂ 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% CO₂ and 37°C in CO₂ incubator (Thermo scientific BB150).



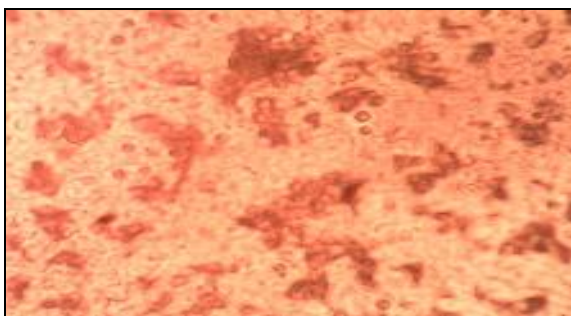
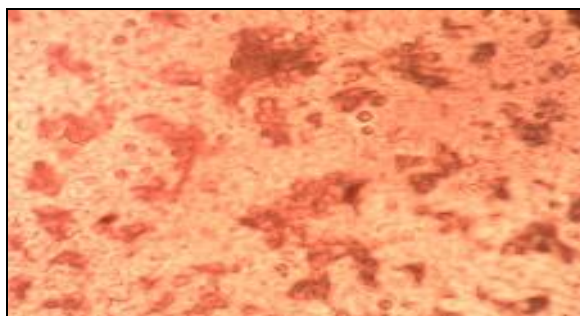
FIG. 3: ELISA PLATE READER

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 ($\mu\text{g/ml}$)	OD	% inhibition	IC ₅₀ ($\mu\text{g/ml}$)
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 $\mu\text{g/ml}$)****FIG. 4(D) CG F TREATED (100 $\mu\text{g/ml}$)****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 IC₅₀ value of 44.44 $\mu\text{g/ml}$.

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 44.44 $\mu\text{g/ml}$, 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 IC₅₀ value of 44.44 $\mu\text{g/ml}$ in a dose-dependent manner against 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 $\mu\text{g/ml}$ 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.

ACKNOWLEDGMENT: We are thankful to the management of Nazareth College of Pharmacy, Othara, Thiruvalla, Kerala, for the help and support and Dr. P. Selvam, Aravindh Herbal Labs Pvt. Ltd, Tamil Nadu.

CONFLICT OF INTEREST: Nil

How to cite this article:

Nisana KS, Sabu AA, Mathew AE, Moncy CK, Gopika B, Jiju V and Abraham E: *In-vitro* anticancer activity of flowers of *Couroupita guianensis* against skin cancer cell line. Int J Pharmacognosy 2022; 9(12): 202-05. doi link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.9\(12\).202-05](http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.9(12).202-05).

This Journal licensed under a Creative Commons Attribution-Non-commercial-Share Alike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)

REFERENCE:

1. Vikas K, Anjali T and Ashwin S: *Couroupita guianensis*: A potential medicinal tree. Int Foundation Inst of Org. Res 2017; 4(10): 30-34.
2. Chandolu SK: Short review on therapeutic uses of *Couroupita guianensis* Aubl. Int Res J Pharm App Sci 2011; 1(1):105-108.
3. Mariappan P, Srinivasan R, Kumarasamy K, Ganesan S, Velayutham T, Thangavel S and Kandasamy K: Antioxidant & anticancer activities of isatin, isolated from the flowers of *Couroupita guianensis* Aubl. Ind J Med Res 2012; 136(5): 822–826.
4. Jeyaraj M and Sathishkumar G: Cytotoxicity effects and antibacterial and of biogenic palladium nanoparticles synthesized using fruit extract of *Couroupita guianensis* Aubl. J of Applied Biomedicine 2018; 16(1): 59-65.