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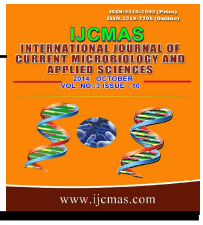

Original Research Article

In-vitro assay for Cytotoxicity activity in ethonolic extract of fruit rind of Couropita *Guianensis aubl*

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Keywords

African Green Monkey Kidney (VERO), MCF-7 (GDC055,-bromide)

A B S T R A C T

Couroupita guianensis possesses antibiotic, antifungal, antiseptic and analgesic qualities). The present work was undertaken to know more about CELL LINE CULTURE to ensure its usage in Pharmaceutical industries. Methanolic extract of *C.guianensis* flower produced central inhibitory effects in mice. The extract significantly reduced spontaneous motor activity in dose dependent manner which gives indications of the level of excitability of the central nervous system, and this decrease may be closely related to sedation resulting from depression of the central nervous system. To emphasizes the pharmacology study of fruit rind ethanolic extract through cell line cultures, acute toxicity and anticancerous activity Human breast cancer MCF – 7 (GDC055), Human colon adenocarcinoma (GDC033) and African Green monkey kidney (VERO) cell lines were obtained from national centre for cell sciences Pune (NCCS).

Introduction

Globally, cancer represents a substantial burden of disease in the community. Every year over 200,000 people are diagnosed with cancer in the United Kingdom only, and approximately 120,000 die as an aftermath of the disease (Department of Health 2000). According to the international Agency for Research on cancer 2002. Cancer killed > 6.7 million people around the world and another 10.9 million new cases were diagnosed (Newman *et al.*, 2003). It's time people stopped living in the illusion that they can't be afflicted with cancer. Cancer is climbing the Indian graph due to rapid lifestyle changes, adding nearly a million new cases every year.

"We have one million new cancer cases coming up every year in India. In the last decade, lifestyle related causes have increased our susceptibility to the disease — it's now time to drop the attitude – 'how me'," P.K. Julka, professor of clinical oncology at the All India Institute of Medical Sciences (AIIMS) (JUNE, 2013).

Also the extract did not abolish the flexor and extensor when administered at a dose of

2000mg/kg for acute toxicity study (Gupta, 2012). To evaluate the safety of a new drug in healthy animals or to assess treatment benefits in liver cancer, patients with outmost safety and compare a new drug against placebo (Dummy medication). Several key guidelines regarding clinical trials have been constructed to ensure that an animal's safety. Before testing with the animals the drugs were tested in various cell line cultures by Pilot study.

The cells were maintained in minimal essential media (MEM) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100μg/mL) in a humidified atmosphere of 50ug/mL CO2 at 37°C. After 48 hours of inculcation, 1μL of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4 hours. The medium with MTT was then flicked off and the formed Formazan crystals were solubilized in 100μL of DMSO and then measured the absorbance at 57m nm using micro plate reader (Mosmann, 1983).

The % cell inhibition was determined using the formula. % cell inhibition = 100 – Abs (sayde) Abs (control) x100. Non linear regression graph was plotted between % cell inhibition and log to concentration and Ic50 was determined using graph pad Prism Software.

Human Breast cancer MCF-7 (GDC055), Human Colon Adenocarcinoma (GDC033) and African Green Monkey Kidney (VERO) cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 50 μg/ml CO_2 at 37 °C. MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

The Cytotoxicity of samples on MCF-7 cells, HT 29 and VERO was determined by the MTT assay (Mosmann, 1983). Cells (1×10^5/well) were plated in 100 μl of medium/well in 96-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20μl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of human breast cancer cells was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100%.

Results and Discussion

Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for 50% inhibition of viability (IC50) was

determined graphically. The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanksThe effect of the samples on the proliferation of human breast cancer cells was expressed as the 83% cell viability, using the following formula: % cell viability = A570 of treated cells / A570 of control cells × 100%.

Drug toxicity (anticancer compound) in in-vitro cell experiment, MTT assay is the most common reaction in the cell culture procedure. Production of monoclonal antibodies requires production of pharmaceutical drugs and study of the effects of toxins and pollutants in the drug using cell lines.

Figure.1

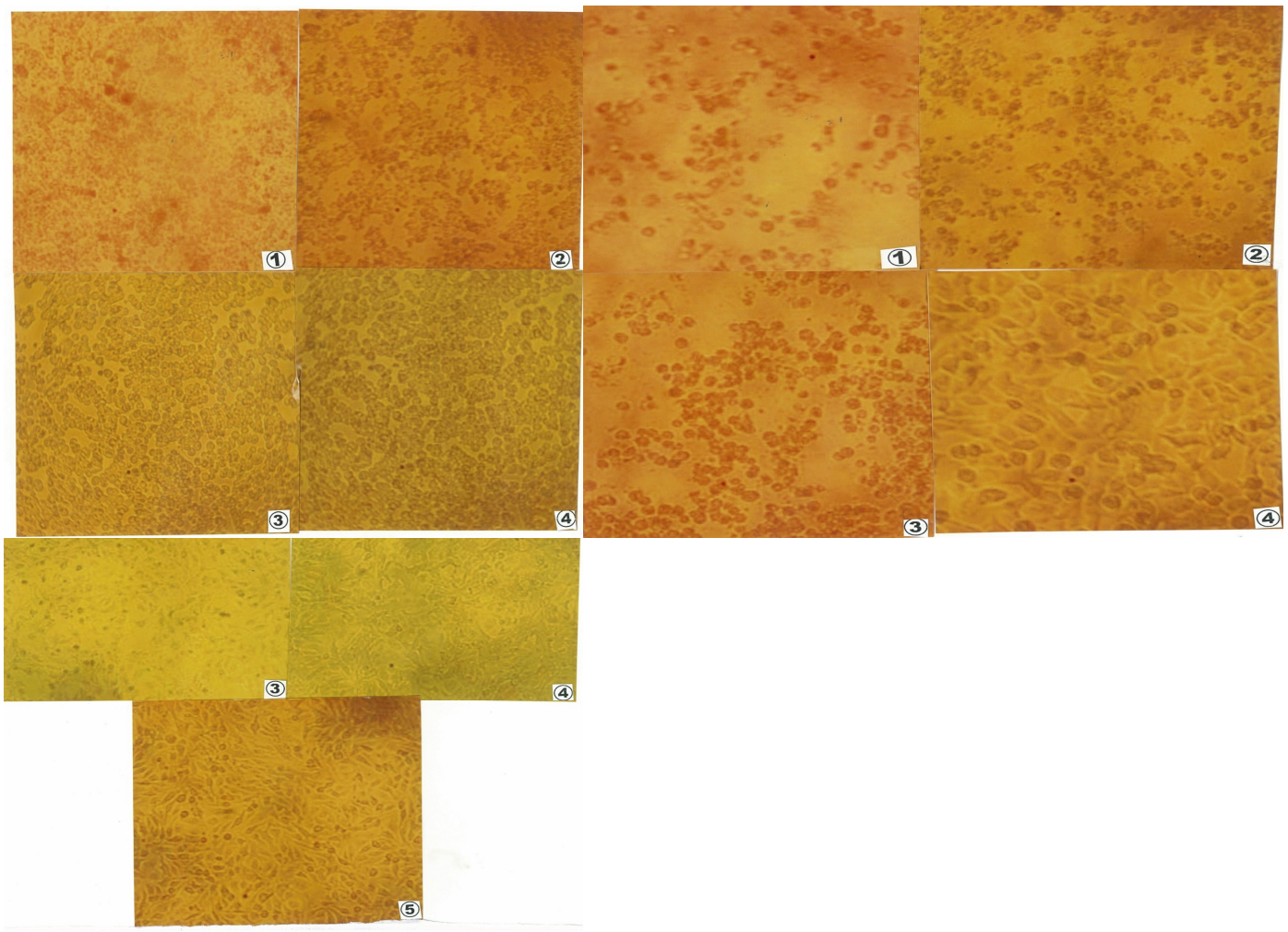


Figure.2

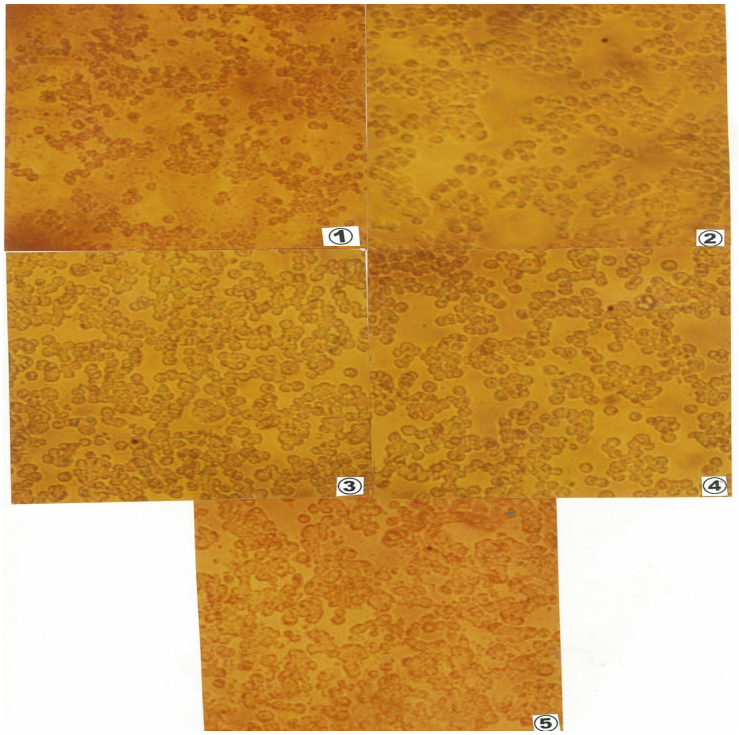
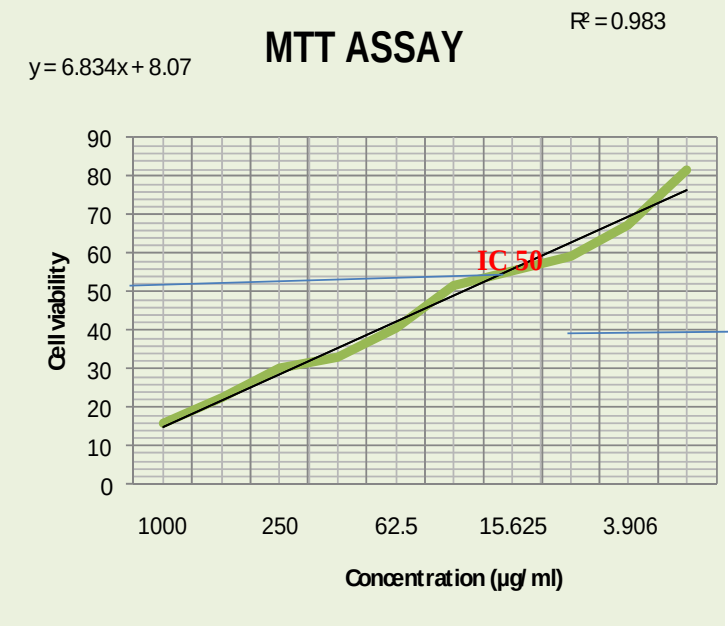
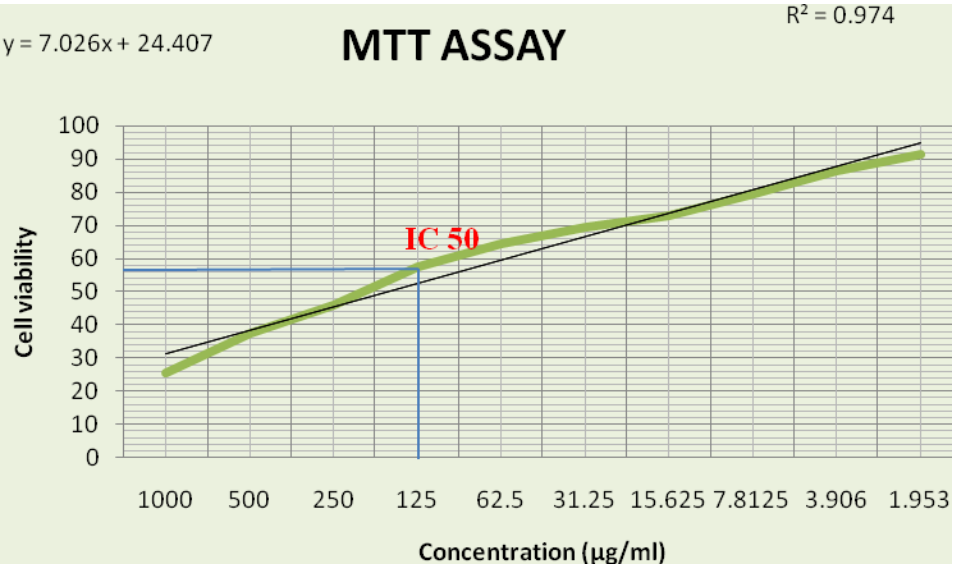


Figure.3

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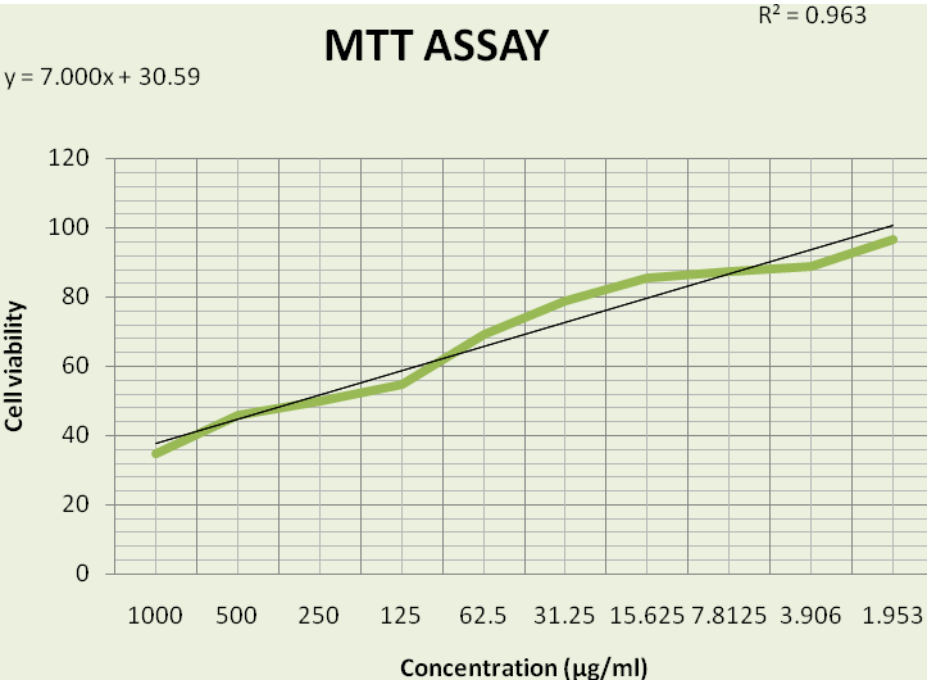
R² = 0.974
MTT ASSAY
IC 50
Cell viability
100
90
80
70
60
50
40
30
20
10
0
1000
500
250
125
62.5
31.25
15.625
7.8125
3.906
1.953
Concentration (µg/ml)

Figure.4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R² = 0.963
MTT ASSAY
Cell viability
120
100
80
60
40
20
0
1000
500
250
125
62.5
31.25
15.625
7.8125
3.906
1.953
Concentration (µg/ml)

Figure.5

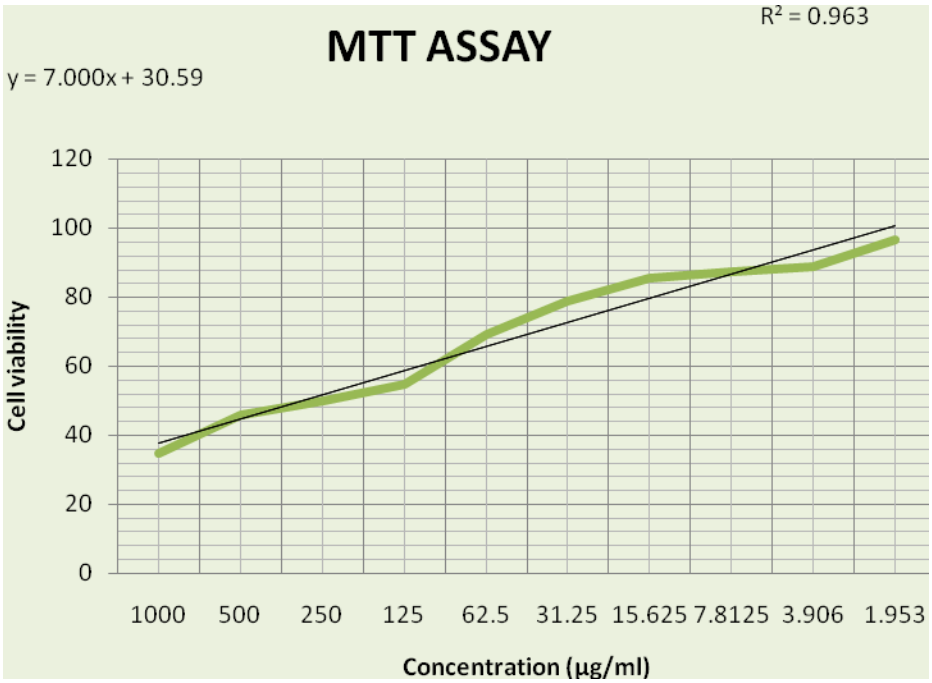

Table.1 MTT assay of *Couroupita guianensis* on Hep G_2 cell lines

S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	1000	Neat	15.79
2.	500	1:1	22.37
3.	250	1:2	30.26
4.	125	1:4	32.89
5.	62.5	1:8	40.79
6.	31.25	1:16	51.32
7.	15.625	1:32	55.26
8.	7.8125	1:64	59.21
9.	3.906	1:128	67.10
10.	1.953	1:256	81.58
11.	Cell control	-	100

Table.2 MTT assay on ethanolic extract of fruit rind of *Couroupita guianensis* on Vero cell lines

S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	1000	Neat	34.51
2.	500	1:1	45.67
3.	250	1:2	49.80
4.	125	1:4	54.44
5.	62.5	1:8	69.01
6.	31.25	1:16	78.72
7.	15.625	1:32	85.61
8.	7.8125	1:64	87.55
9.	3.906	1:128	89.01
10.	1.953	1:256	96.71
11.	Cell control	-	100

Table.3 MTT assay on ethanolic extract of fruit rind of *Couroupita guianensis* on MCF 7 cell lines

S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	1000	Neat	12.33
2.	500	1:1	17.88
3.	250	1:2	23.33
4.	125	1:4	27.89
5.	62.5	1:8	35.11
6.	31.25	1:16	39.08
7.	15.625	1:32	45.63
8.	7.8125	1:64	53.99
9.	3.906	1:128	67.89
10.	1.953	1:256	71.56
11.	Cell control	-	100

Table.4 MTT assay on ethanolic extract of fruit rind of *Couroupita guianensis* on HT 29 cell lines

S.No.	Concentration (µg /ml)	Dilutions	Cell viability
1.	100	Neat	18.85±1.32
2.	50	1:1	32.45±0.95
3.	25	1:2	45.65±0.85
4.	12.5	1:4	58.32±0.45
5.	6.25	1.8	67.95±1.35
6.	3.125	1:16	78.95±1.49
7.	1.56	1:32	91.15±0.385
8.	Cell control	-	100

In our cell line culture the ethanolic extract of fruit rind showed activity in the MTT assay using four tumor cell lines, in HEP G2, 62.5 μg / ml., Vero Cell line, 62.5 μg / ml., Breast cancer cell line 15.625μg / ml. and in HT 29-Colon cancer cell line**,** 62.5 μg / ml. indicating the presence of 83% cytotoxic compounds in the ethanolic extract of fruit rind, hence IC 50 values for our tested cell lines. Prabhu and Ravi, 2012 has reported the viability of cancer cells after incubation with different concentration. The incubation with different concentration of methanol extracts (1.75, 2.1, 2.45, 2.60 and 3.0 μg/ml.) Affected the viability of human cancer cell lines from the methanolic extract of fresh flowers, and dried flowers also showed cytotoxic effect on the Hel.a, Hep G_2 and N_1H_1, 3T3 cancer cell lines.

Methanol extract was more active towards Hel.a cell lines, when compared with Hep G_2 and N_1H_1, 3T3 cell lines. Premanathan *et al.*, 2012 have reported in **flowers** the cytotoxicity of Isatin was studied in Human promyelocytic leukemia HL60 cells. The 50 percent cytotoxicity (cc50) value was determined after five days of exposure. Isatin showed cytotoxicity in dose-dependent at manner with cc50 of 2.94 μg / ml. Fluorouracil was used as positive control with cc50 of 0.07μm.

In this study determined that the ethanolic extract concentration decreases the cytotoxicity effect increases in all the human and animal cell lines in vitro. Concomitantly, we observed an improvement in proliferation rates and changes in the morphology of contaminated mammalian cells after treatment with this phytodrug.

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