



**PONDICHERRY CENTRE FOR BIOLOGICAL SCIENCE AND
EDUCATIONAL TRUST**

Reg.No.2840/B4/2016 Pondicherry

GST IN: 33AADTP1541L1ZL

SUSTAINABLE RESEARCH FOR BETTERMENT OF MANKIND

CERTIFICATE OF ANALYSIS

PCBSET/2021-2022/RN00130_1

Date: 21/03/2022

User information: **Dr.Joe Antony. J,**
Nanome Consulting,
22/11, Shanthi Avenue,
Ramanathapuram,
Kannankurichi,
Salem – 636008
Tamil Nadu,
India

Sample information: Powder

Sample Code: Joe-2

Analysis: Cytotoxicity Assay_HCT-15

Date of sample received: 13/03/2022

Date of Analysis: 15/03/2022

Date of report: 21/03/2022

NITI Aayog



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Methodology:

Cell culture and MTT assay:

The Human Colon Cancer cell line (**HCT-15**) were plated separately using 96 well plates with the concentration of 1×10^4 cells/well in RPMI media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO₂ incubator at 37°C with 5% CO₂. The cells were washed with 200 µL of 1X PBS, then the cells were treated with various test concentration of compound in serum free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37°C for 4 h using CO₂ incubator. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals was dissolved with 100 µL of DMSO and thoroughly mixed. The development of color intensity was evaluated at 570nm. The formazan dye turns to purple blue color. The absorbance was measured at 570 nm using microplate reader.

Reference:

1. Florento L, Matias R, Tuaño E, Santiago K, Dela Cruz F, Tuazon A. Comparison of Cytotoxic Activity of Anticancer Drugs against Various Human Tumor Cell Lines Using In Vitro Cell-Based Approach. Int J Biomed Sci. 2012;8(1):76-80.

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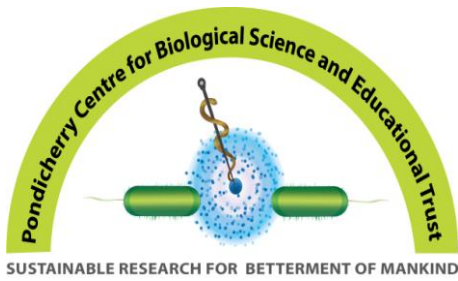
MINISTRY OF MICRO, SMALL & MEDIUM ENTERPRISES
UDYAM REGISTRATION NUMBER - UDYAM-TN-31-000540

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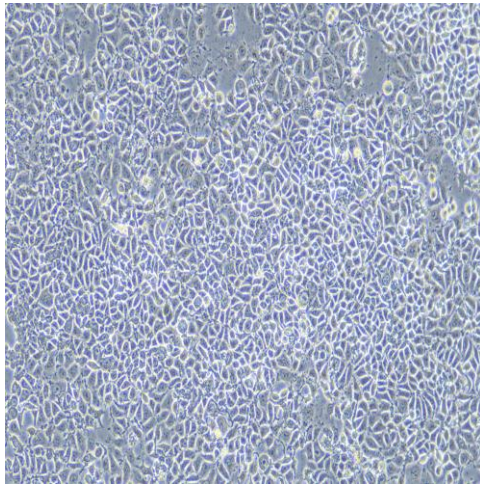
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Results

Control



Sample code: Joe-2

Raw data:

Tested concentration($\mu\text{g/ml}$)	OD at 570nm(triplicate values)		
	500	0.015	0.012
250	0.020	0.018	0.022
100	0.165	0.172	0.175
50	0.200	0.210	0.215
25	0.235	0.242	0.247
Control	0.37	0.364	0.357

Final data

Tested concentration($\mu\text{g/ml}$)	% of Cell Viability			Mean	Standard Deviation	Standard Error
	500	5.77	4.62			
250	7.69	6.92	8.46			
100	63.46	66.15	67.31			
50	76.92	80.77	82.69			
25	90.38	93.08	95.00			
Control	98.08	105.38	96.15			

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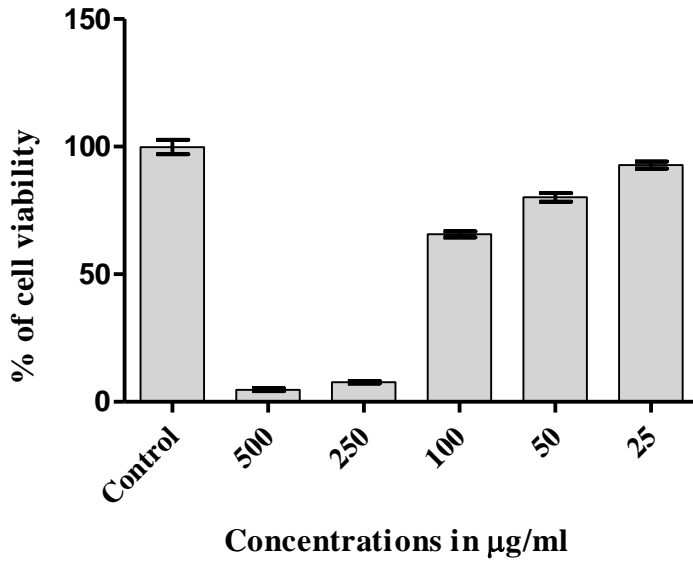


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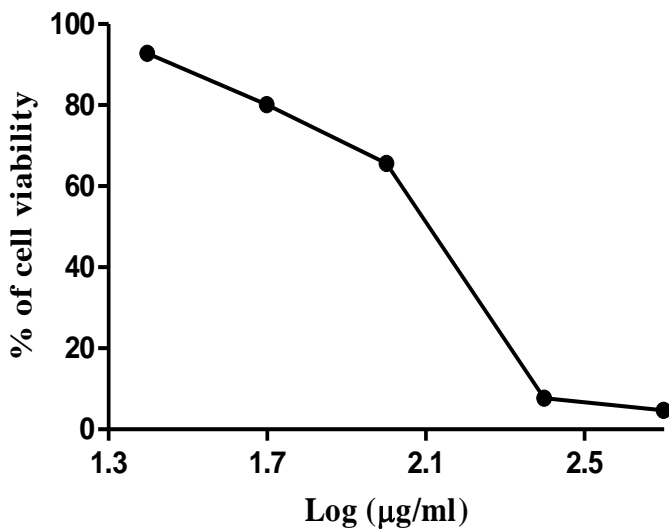
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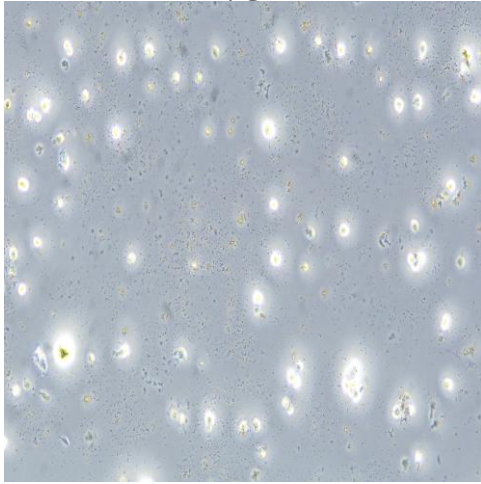


IC50 graph

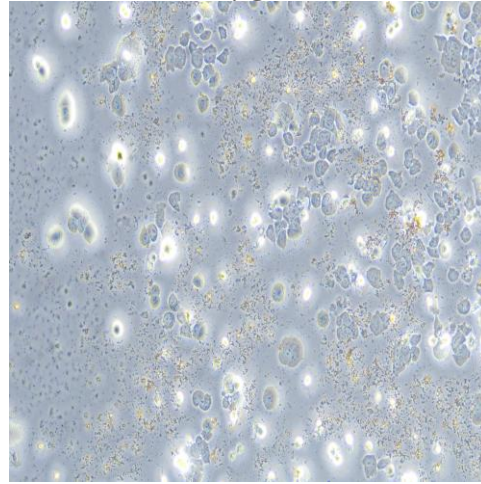


IC50 = $116 \pm 2.1 \mu\text{g/ml}$

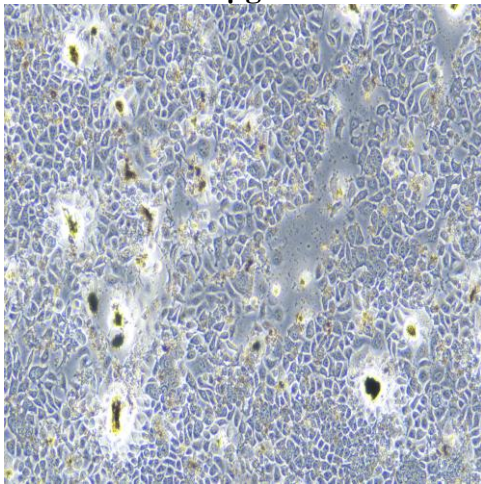
500µg/ml



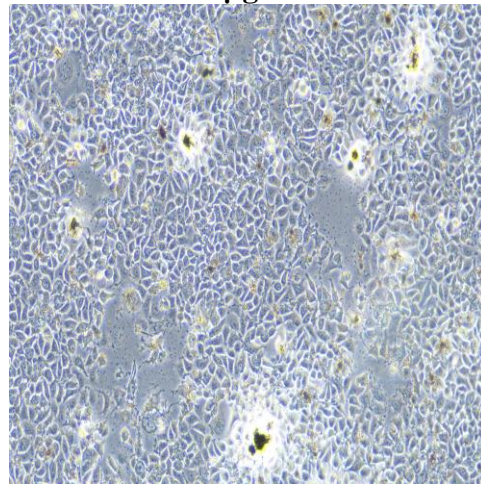
250µg/ml



100µg/ml



50µg/ml



25µg/ml

