

**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\_2

Date: 21/03/2022

**User information:** **Dr.Joe Antony. J,**  
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Kannankurichi,  
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India

**Sample information:** Powder

**Sample Code:** Joe-2

**Analysis:** Cytotoxicity Assay\_L929

**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 Mouse Fibroblast cell line (**L929**) were plated separately using 96 well plates with the concentration of  $1 \times 10^4$  cells/well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO<sub>2</sub> incubator at 37°C with 5% CO<sub>2</sub>. 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 CO<sub>2</sub> 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.

**NITI Aayog**



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MINISTRY OF MICRO, SMALL & MEDIUM ENTERPRISES  
UDYAM REGISTRATION NUMBER - UDYAM-TN-31-0009450

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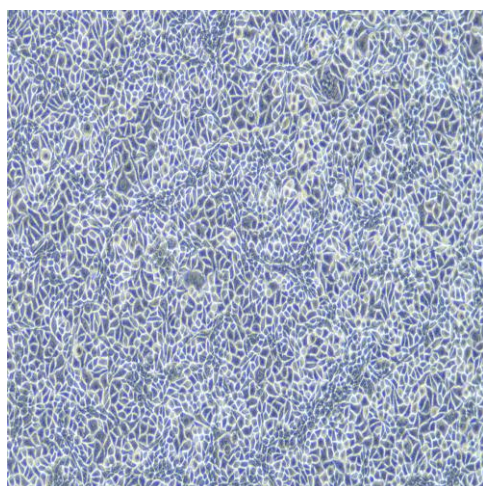
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**Results**

**Control**



**Sample code: Joe-2**

**Raw data:**

Tested concentration( $\mu\text{g/ml}$ )	OD at 570nm(triplicate values)		
	500	0.285	0.297
250	0.300	0.312	0.308
100	0.329	0.333	0.335
50	0.338	0.341	0.337
25	0.342	0.340	0.339
<b>Control</b>	<b>0.370</b>	<b>0.364</b>	<b>0.357</b>

**Final data**

Tested concentration( $\mu\text{g/ml}$ )	% of Cell Viability			Mean	Standard Deviation	Standard Error
	500	83.82	87.35			
250	88.24	91.76	90.59	<b>90.20</b>	1.80	1.04
100	96.76	97.94	98.53	<b>97.75</b>	0.90	0.52
50	99.41	100.29	99.12	<b>99.61</b>	0.61	0.35
25	100.59	100.00	99.71	<b>100.10</b>	0.45	0.26
<b>Control</b>	101.18	100.00	98.82	<b>100.00</b>	1.18	0.68

**NITI Aayog**

**NGO D ARPAN**  
NGO Darpan Unique ID - PY1202010270047

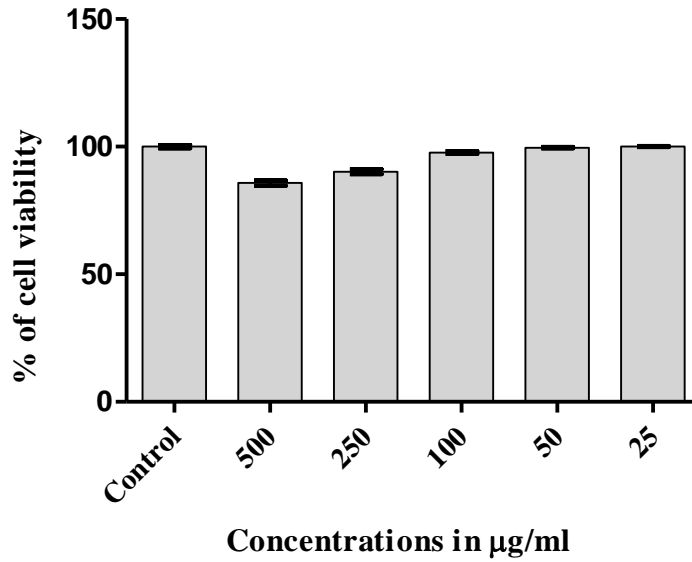
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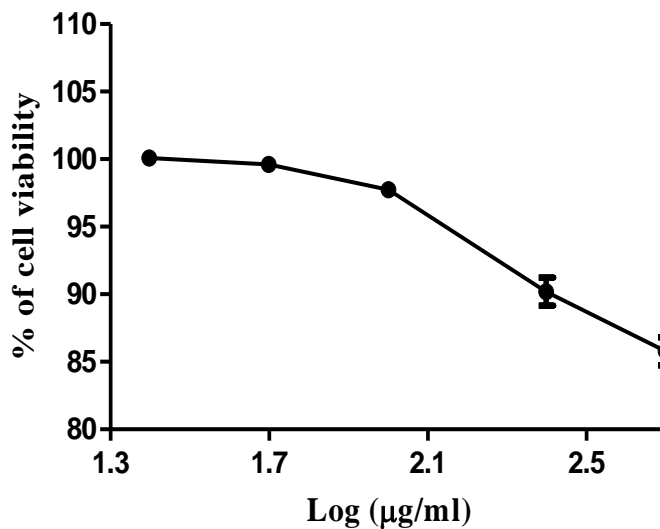
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**MSME**

MINISTRY OF MICRO, SMALL & MEDIUM ENTERPRISES  
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IC50 graph



IC50 = 2596  $\pm$  3.65  $\mu\text{g/ml}$

