

**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/RN00132\_1

Date: 23/03/2022

**User information:** Mrs. J. Manonmani,  
Research Scholar,  
Annamalai University,  
Annamalai Nagar, Chidambaram – 608002,  
Tamil Nadu,  
India

**Sample information:** Powder

**Sample Code:** G.A, S.A, C.A

**Analysis:** Cytotoxicity Assay\_HepG2

**Date of sample received:** 30/01/2022

**Date of Analysis:** 09/03/2022

**Date of report:** 12/03/2022

**NITI Aayog**



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



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### Methodology:

#### Cell culture and MTT assay:

The Human Liver Cancer cell line (**HepG2**) were plated separately using 96 well plates with the concentration of  $1 \times 10^4$  cells/well in MEM 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, Tũaño 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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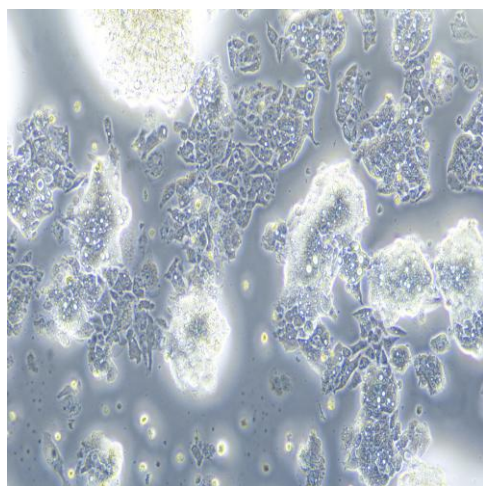
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**Results**

**Control**



Sample code: G.A

Raw data:

Tested concentration( $\mu\text{g/ml}$ )	OD at 570nm(triplicate values)		
500	0.009	0.011	0.010
250	0.015	0.021	0.018
100	0.020	0.030	0.025
50	0.080	0.074	0.071
25	0.152	0.148	0.145
<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	2.47	3.02	2.75	<b>2.75</b>	0.27	0.16
250	4.12	5.77	4.95	<b>4.95</b>	0.82	0.48
100	5.49	8.24	6.87	<b>6.87</b>	1.37	0.79
50	21.98	20.33	19.51	<b>20.60</b>	1.26	0.73
25	41.76	40.66	39.84	<b>40.75</b>	0.96	0.56
<b>Control</b>	101.65	100.00	98.08	<b>99.91</b>	1.79	1.03

**NITI Aayog**

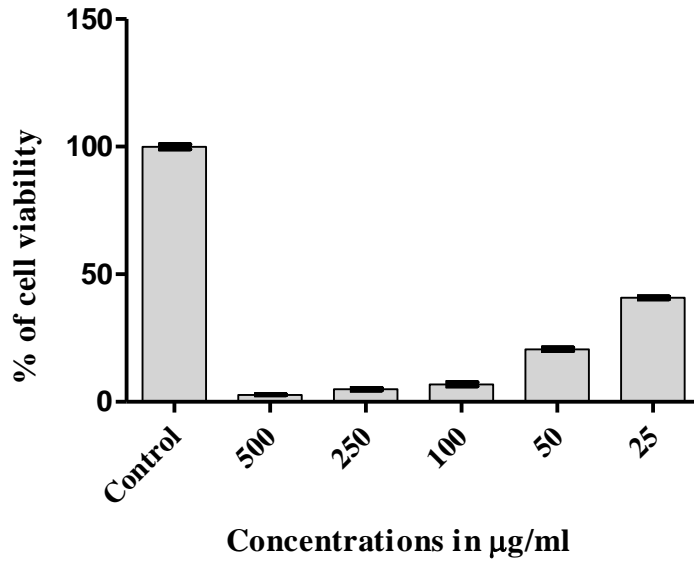


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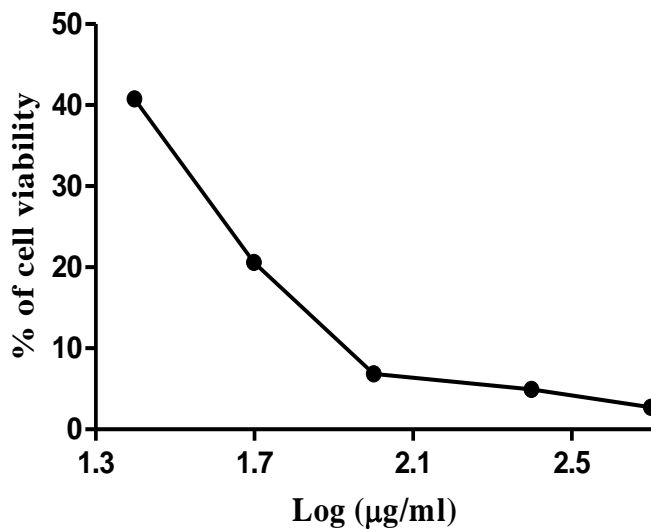
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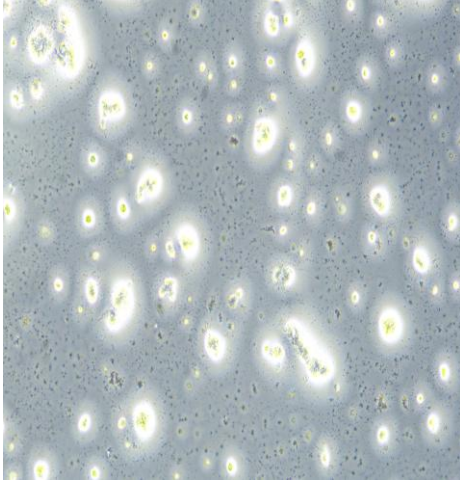


IC50 graph

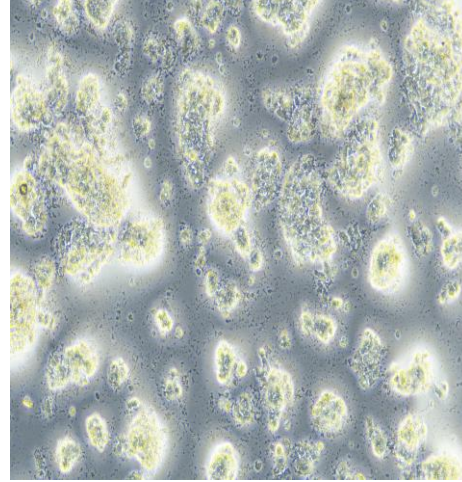


IC50 = 19.09  $\pm$  0.01  $\mu\text{g/ml}$

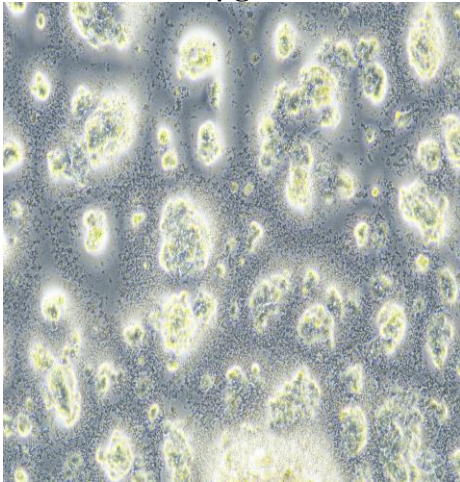
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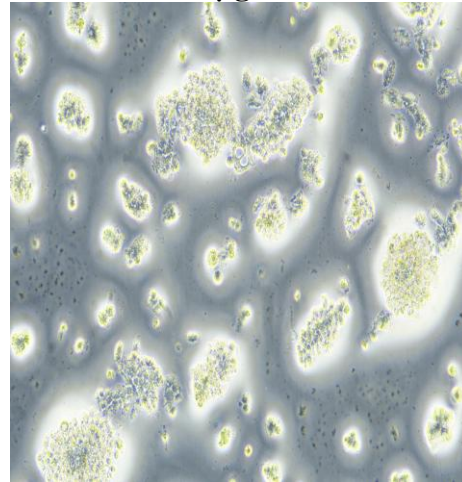
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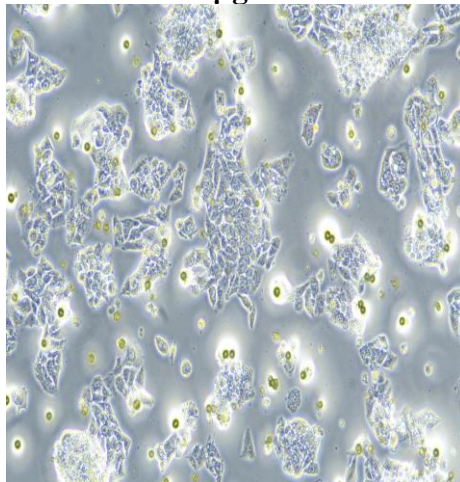
**100µg/ml**



**50µg/ml**



**25µg/ml**



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Sample code: S.A

Raw data:

Tested concentration( $\mu\text{g/ml}$ )	OD at 570nm(triplicate values)		
	500	0.006	0.004
250	0.009	0.011	0.008
100	0.017	0.014	0.015
50	0.028	0.027	0.032
25	0.038	0.042	0.045
<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	1.65	1.10			
250	2.47	3.02	2.20	<b>2.56</b>	0.42	0.24
100	4.67	3.85	4.12	<b>4.21</b>	0.42	0.24
50	7.69	7.42	8.79	<b>7.97</b>	0.73	0.42
25	10.44	11.54	12.36	<b>11.45</b>	0.96	0.56
<b>Control</b>	101.65	100.00	98.08	<b>99.91</b>	1.79	1.03

NITI Aayog

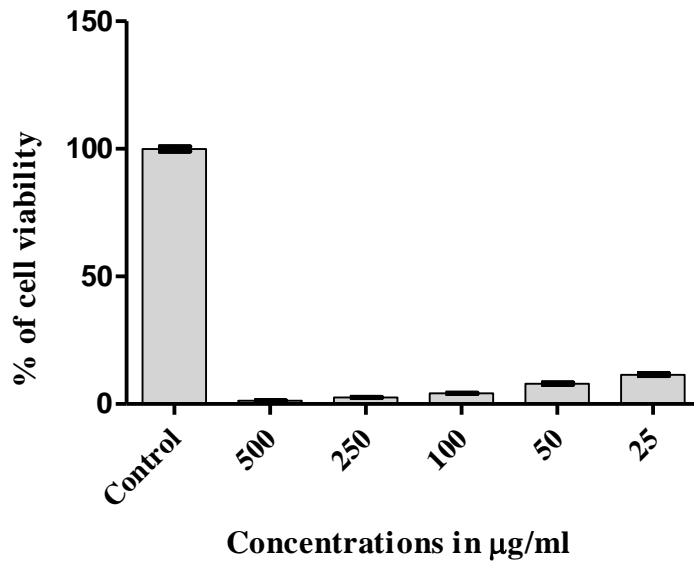


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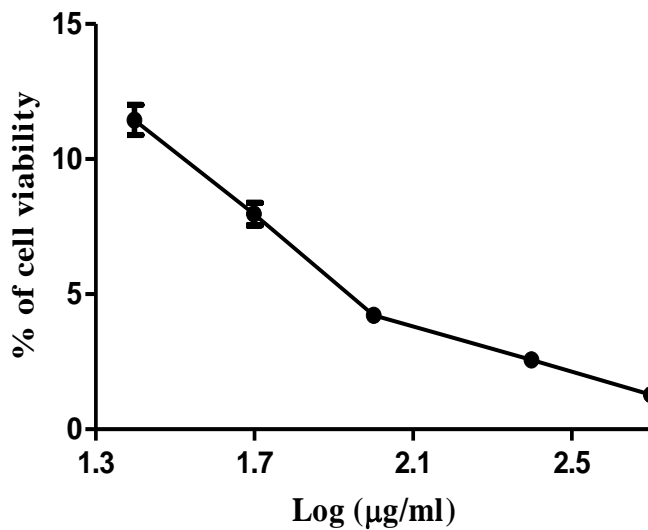
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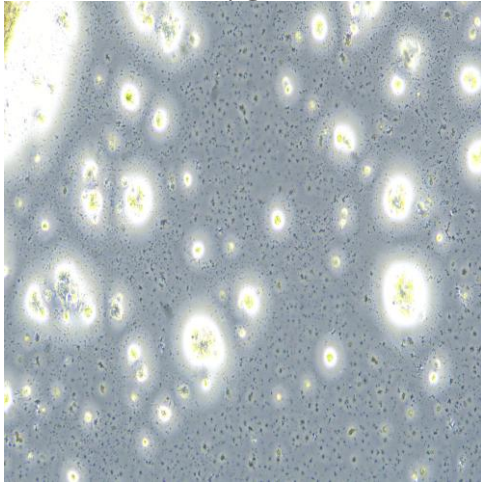


IC50 graph

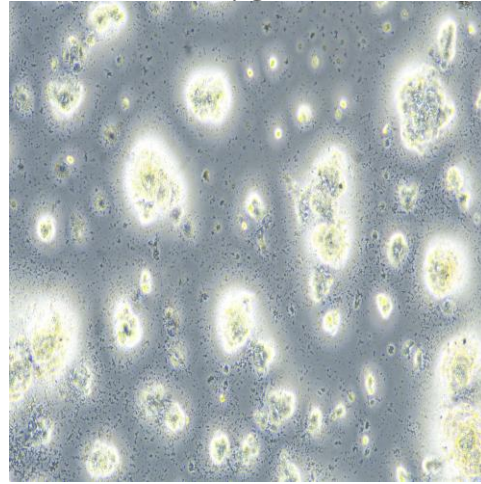


IC50 =  $1.51 \pm 0.36$   $\mu\text{g/ml}$

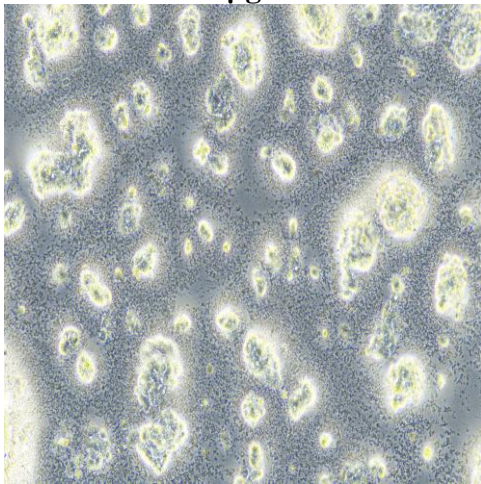
**500µg/ml**



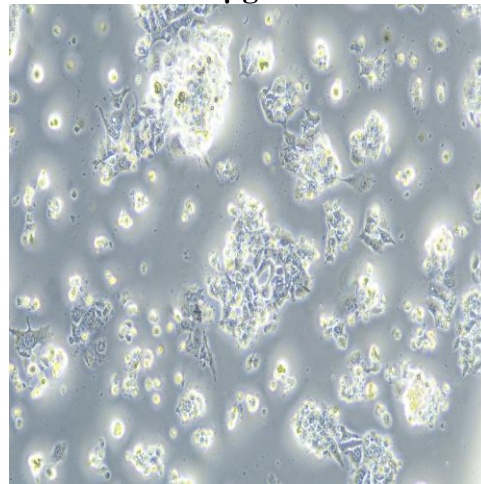
**250µg/ml**



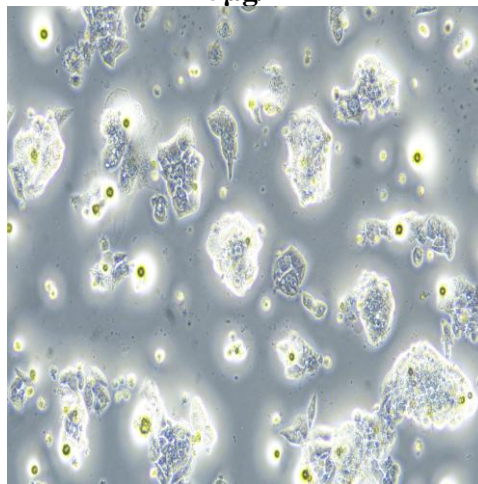
**100µg/ml**



**50µg/ml**



**25µg/ml**

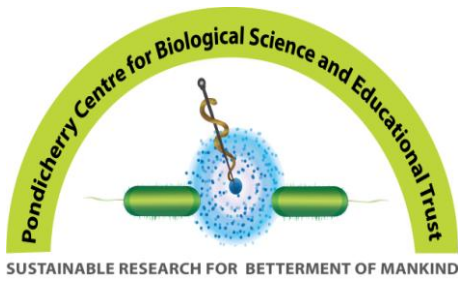


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Sample code: C.A

Raw data:

Tested concentration( $\mu\text{g/ml}$ )	OD at 570nm(triplicate values)		
500	0.003	0.008	0.007
250	0.014	0.017	0.011
100	0.020	0.025	0.022
50	0.050	0.060	0.055
25	0.110	0.105	0.115
<b>Control</b>	<b>0.37</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	0.82	2.20	1.92	<b>1.65</b>	0.73	0.42
250	3.85	4.67	3.02	<b>3.85</b>	0.82	0.48
100	5.49	6.87	6.04	<b>6.14</b>	0.69	0.40
50	13.74	16.48	15.11	<b>15.11</b>	1.37	0.79
25	30.22	28.85	31.59	<b>30.22</b>	1.37	0.79
<b>Control</b>	101.65	100.00	98.08	<b>99.91</b>	1.79	1.03

**NITI Aayog**

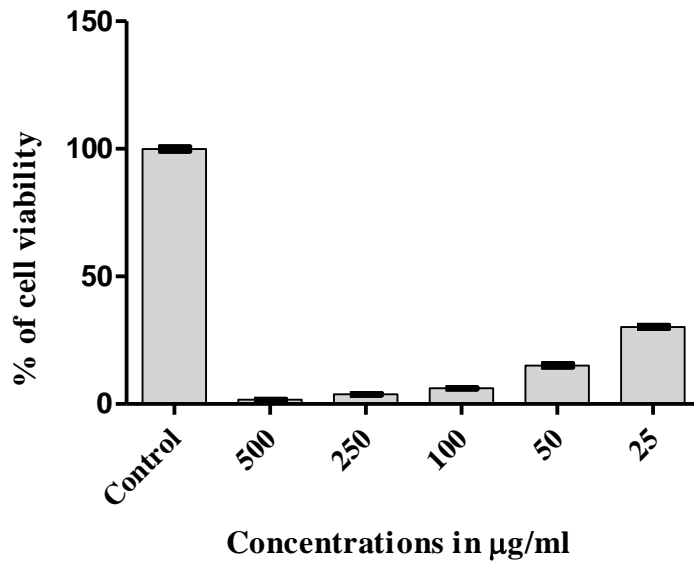
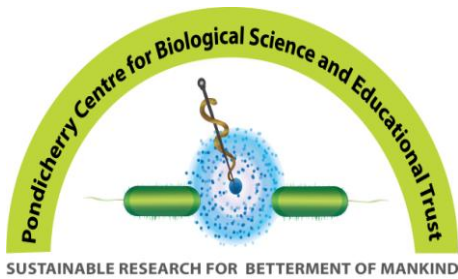


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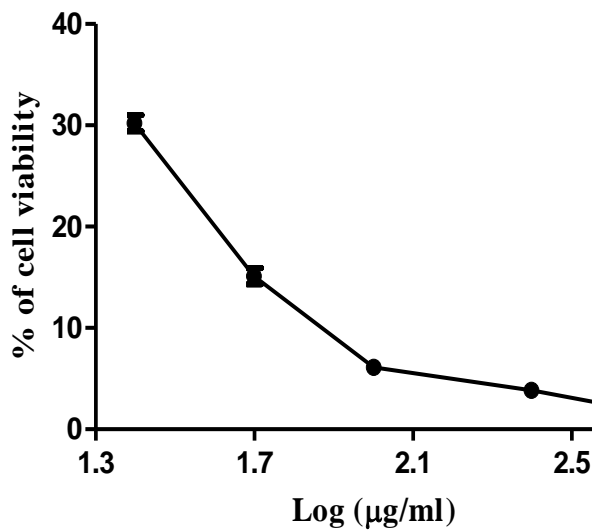
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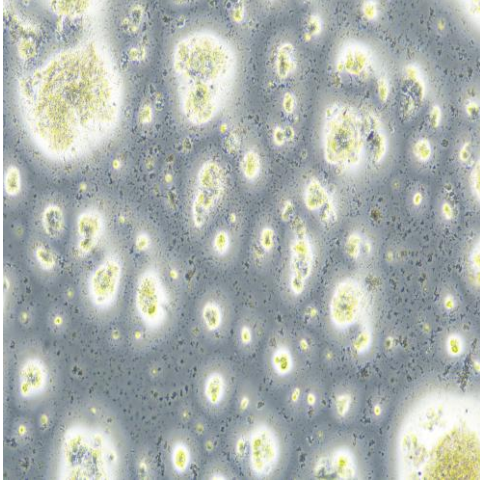


IC50 graph

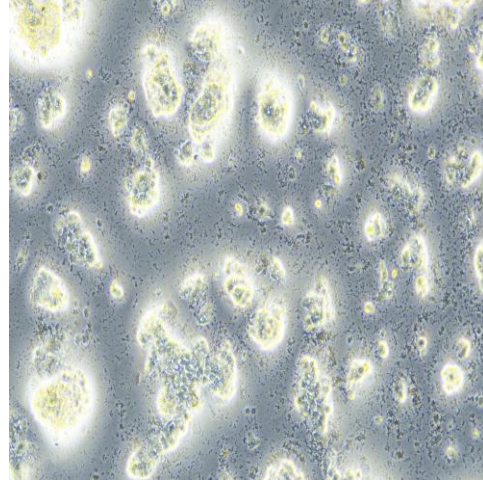


IC50 =  $12.71 \pm 1.51$   $\mu\text{g/ml}$

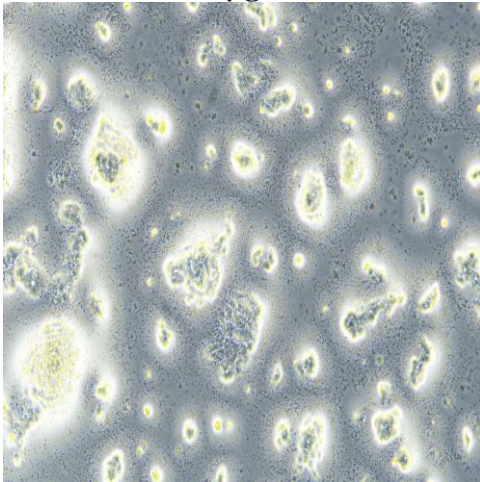
**500µg/ml**



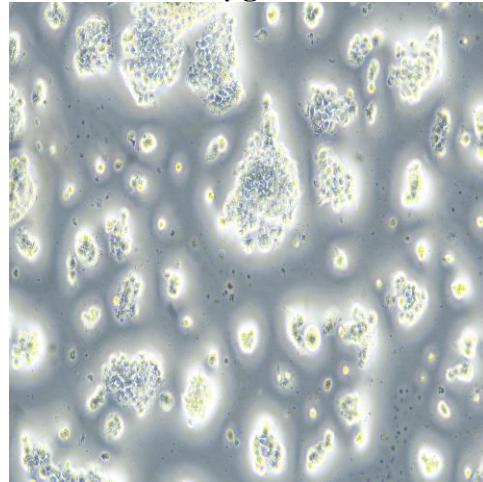
**250µg/ml**



**100µg/ml**



**50µg/ml**



**25µg/ml**

