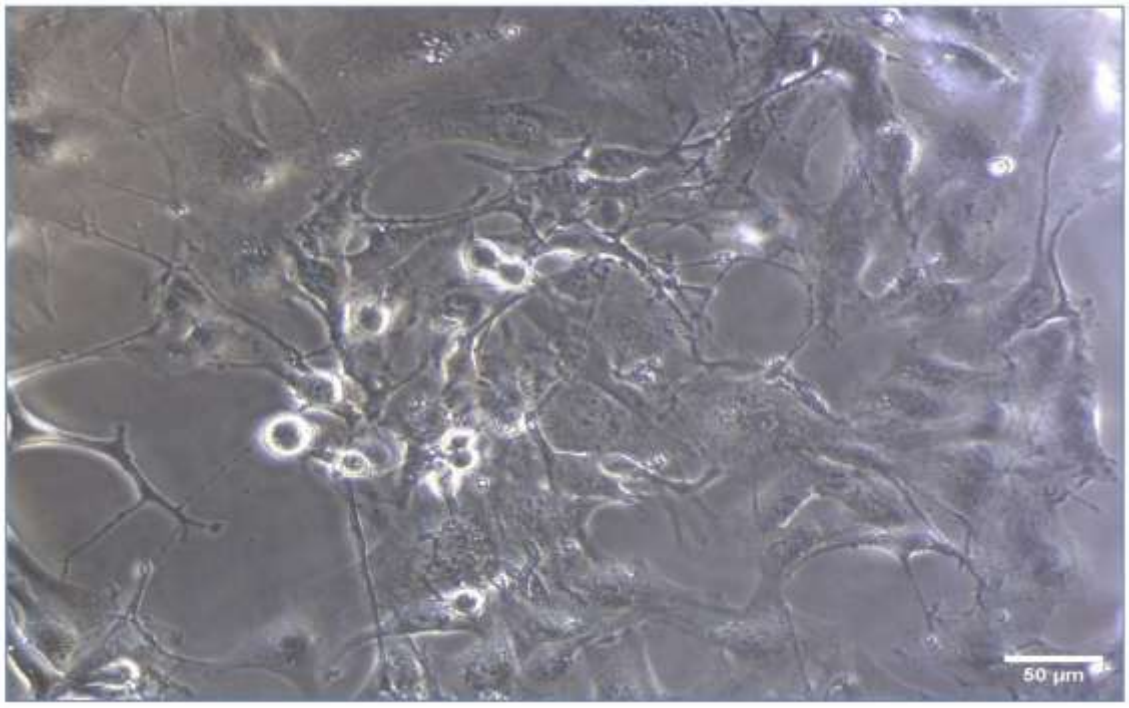
MTT assay

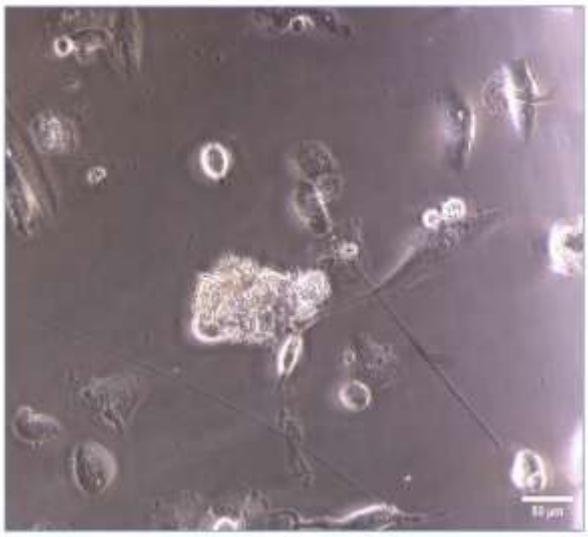
The ethanolic extract of *B. crispa* was evaluated against the skin (A-431, HaCaT) and brain (U87-MG) cancer cell lines at doses ranging from 25 to 800 µg/ml. BE showed cytotoxicity against A-431 and U87-MG with an IC_{50} of 766.675 µg/mL, and 744.23 µg/mL respectively. In HaCaT cell lines, IC_{50} was not achieved even at the highest concentration of 800 µg/mL. *B. crispa* ethanolic extract inhibited the proliferation of tumor cells in a dose-dependent manner in A-431 and U87-MG; the detailed results are represented in Figure 5(B-D). Isolated fraction (B22-B24) was found to have IC_{50} of 485.04 µg/mL and showed cytotoxicity against A-431 cell lines when compared with untreated and standard in MTT assay. The cell lines were tested over a range of 15.625 to 500 µg/mL as shown in Figure 5B(c). The isolated fraction (B22-B24) significantly ($P<0.01$) inhibited the proliferation of A-431 in a dose-dependent manner.

Table 1. MTT data analysis: U-87MG cell line with standard drug (Docetaxel)

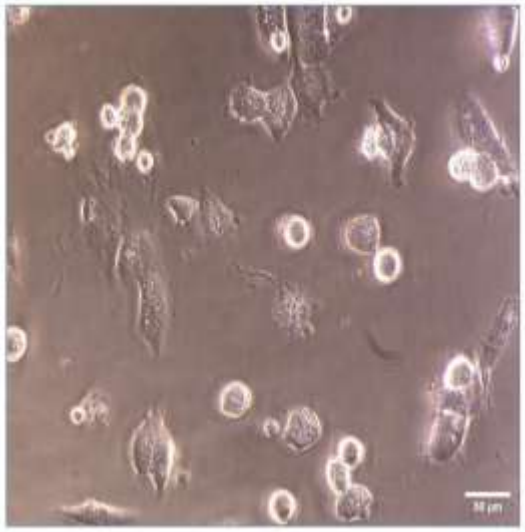
	Test concentration µg/mL							
	Blank	**Untreated**	**25**	**50**	**100**	**200**	**400**	**800**
Reading 1	0.004	0.721	0.332	0.319	0.194	0.085	0.047	0.053
Reading 2	0.007	0.709	0.316	0.283	0.238	0.091	0.086	0.027
Reading 3	0.008	0.746	0.346	0.248	0.183	0.114	0.07	0.031
Mean OD	0.006	0.725	0.331	0.283	0.205	0.097	0.068	0.037
Mean OD-Mean Blank		0.7190	0.3250	0.2770	0.1987	0.0903	0.0613	0.0307
Standard deviation		0.0189	0.0150	0.0355	0.0291	0.0153	0.0196	0.0140
Standard error		0.0109	0.0087	0.0205	0.0168	0.0088	0.0113	0.0081
% Standard error		1.5158	1.2054	2.8507	2.3370	1.2292	1.5742	1.1242
% Viability		100	45.20	38.53	27.63	12.56	8.53	4.27
IC_{50}= 16.77 µg/mL								



U87-MG
Untreated



Docetaxel 25 µg/mL



Docetaxel 50 µg/mL

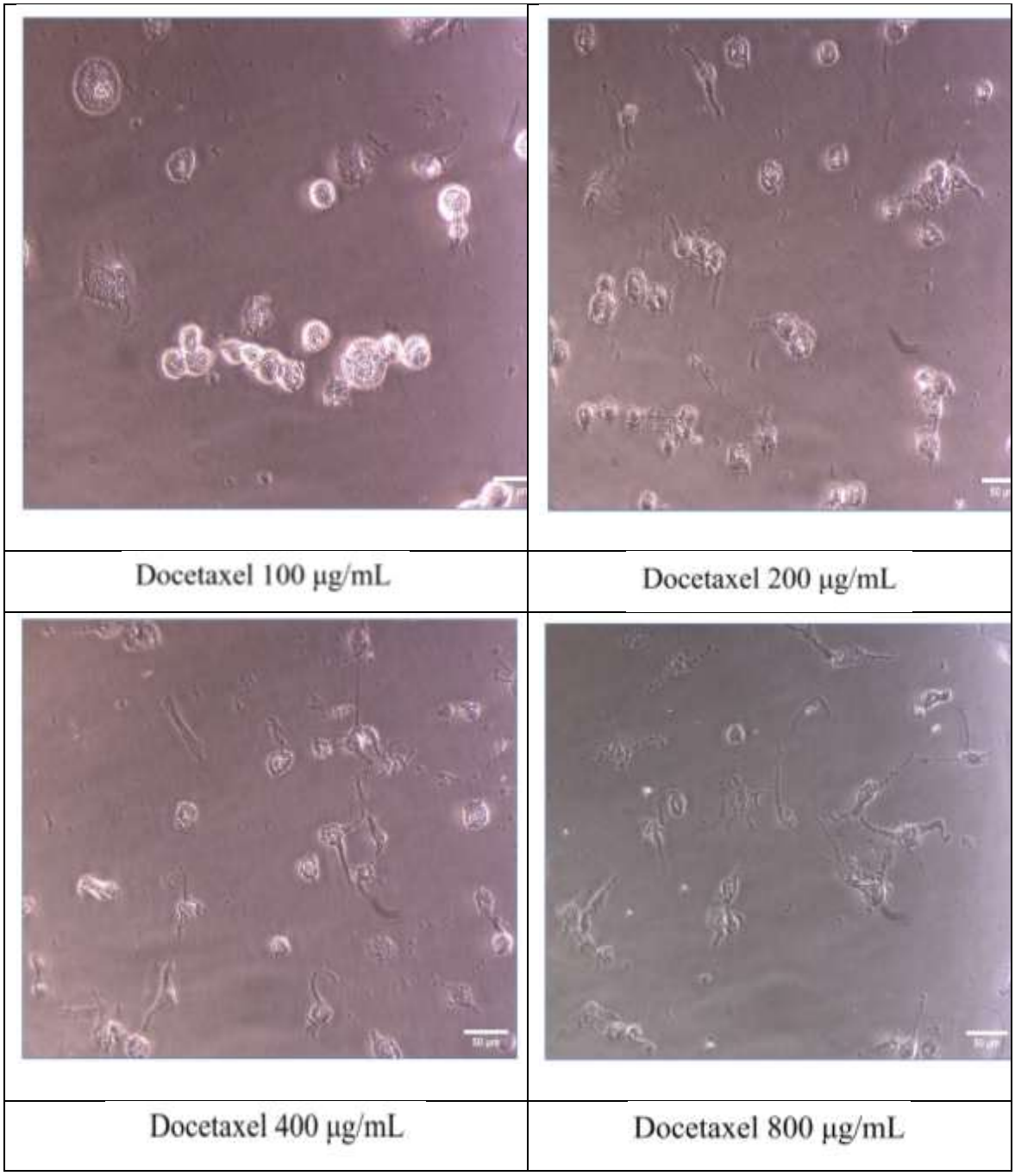


Figure : Treatment of U87-MG cell line with standard drug (docetaxel)

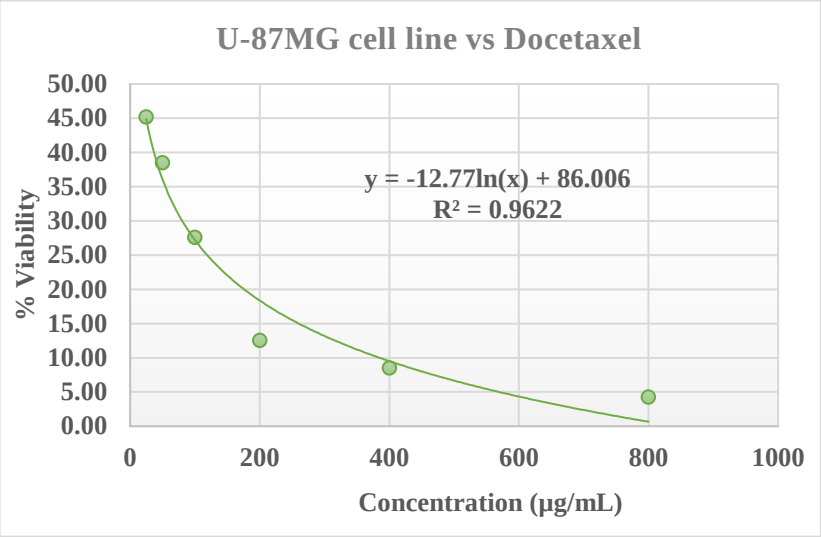


Figure: Represents R^2 value-Plot between % viability and concentration of U 87-MG cell lines vs Docetaxel

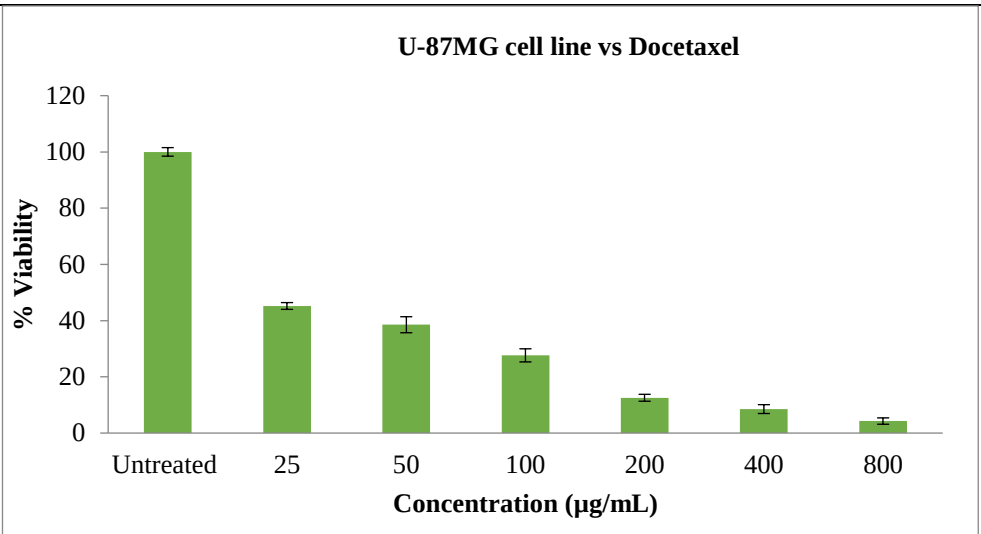


Figure Represents plot between % viability and concentration of U 87-MG cell lines vs Docetaxel

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Table 1. MTT data analysis: U-87MG cell line vs BE

	Test concentration µg/mL							
	Blank	**Untreated**	**25**	**50**	**100**	**200**	**400**	**800**
Reading 1	0.004	0.721	0.749	0.596	0.604	0.543	0.404	0.304
Reading 2	0.007	0.709	0.699	0.606	0.584	0.546	0.433	0.363
Reading 3	0.008	0.746	0.704	0.623	0.606	0.528	0.472	0.321
Mean OD	0.006	0.725	0.717	0.608	0.598	0.539	0.436	0.329
Mean OD-Mean Blank		0.7190	0.7110	0.6020	0.5917	0.5327	0.4300	0.3230
Standard deviation		0.0189	0.0275	0.0137	0.0122	0.0096	0.0341	0.0304
Standard error		0.0109	0.0159	0.0079	0.0070	0.0056	0.0197	0.0175
% Standard error		1.5158	2.2113	1.0961	0.9769	0.7744	2.7400	2.4387
% Viability		100	98.89	83.73	82.29	74.08	59.81	44.92
IC_{50}= 744.23 µg/mL								

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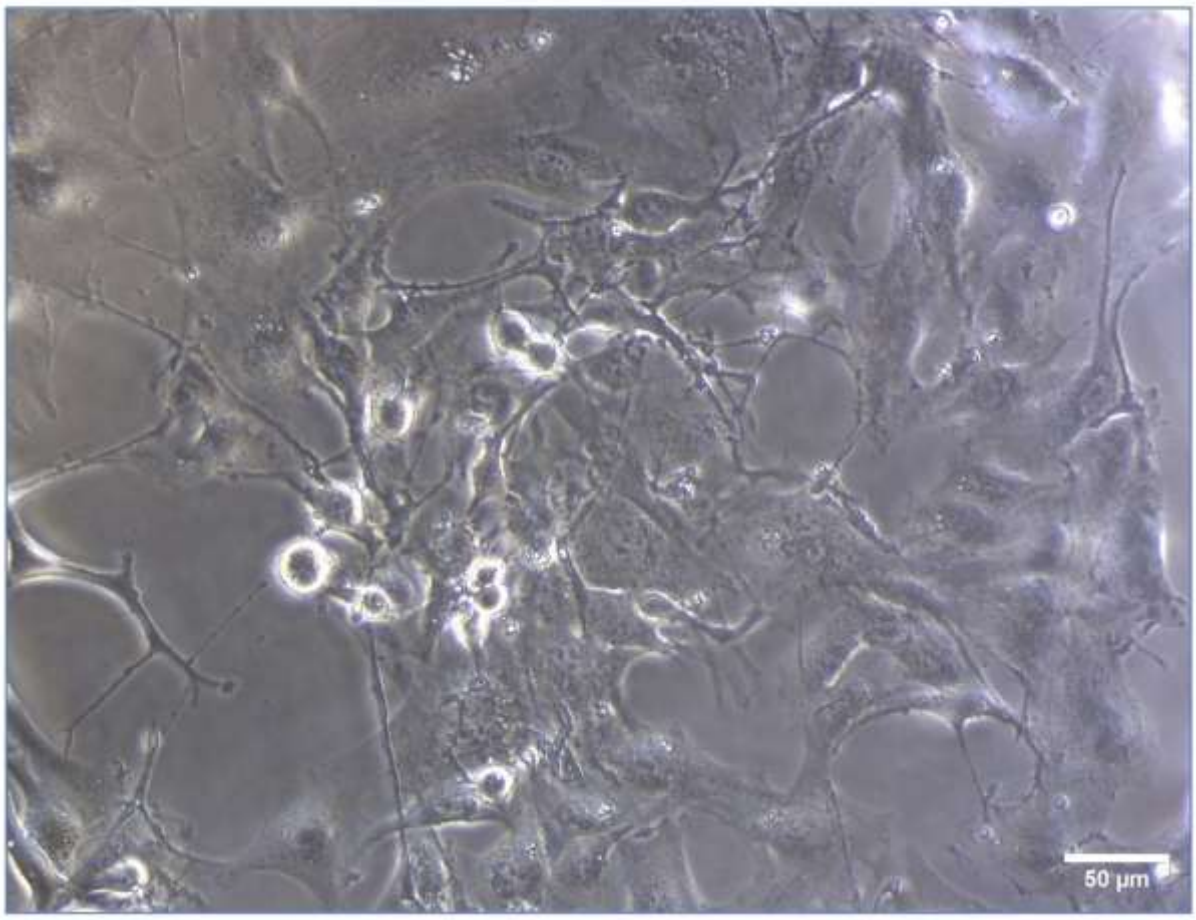

U87-MG
Untreated

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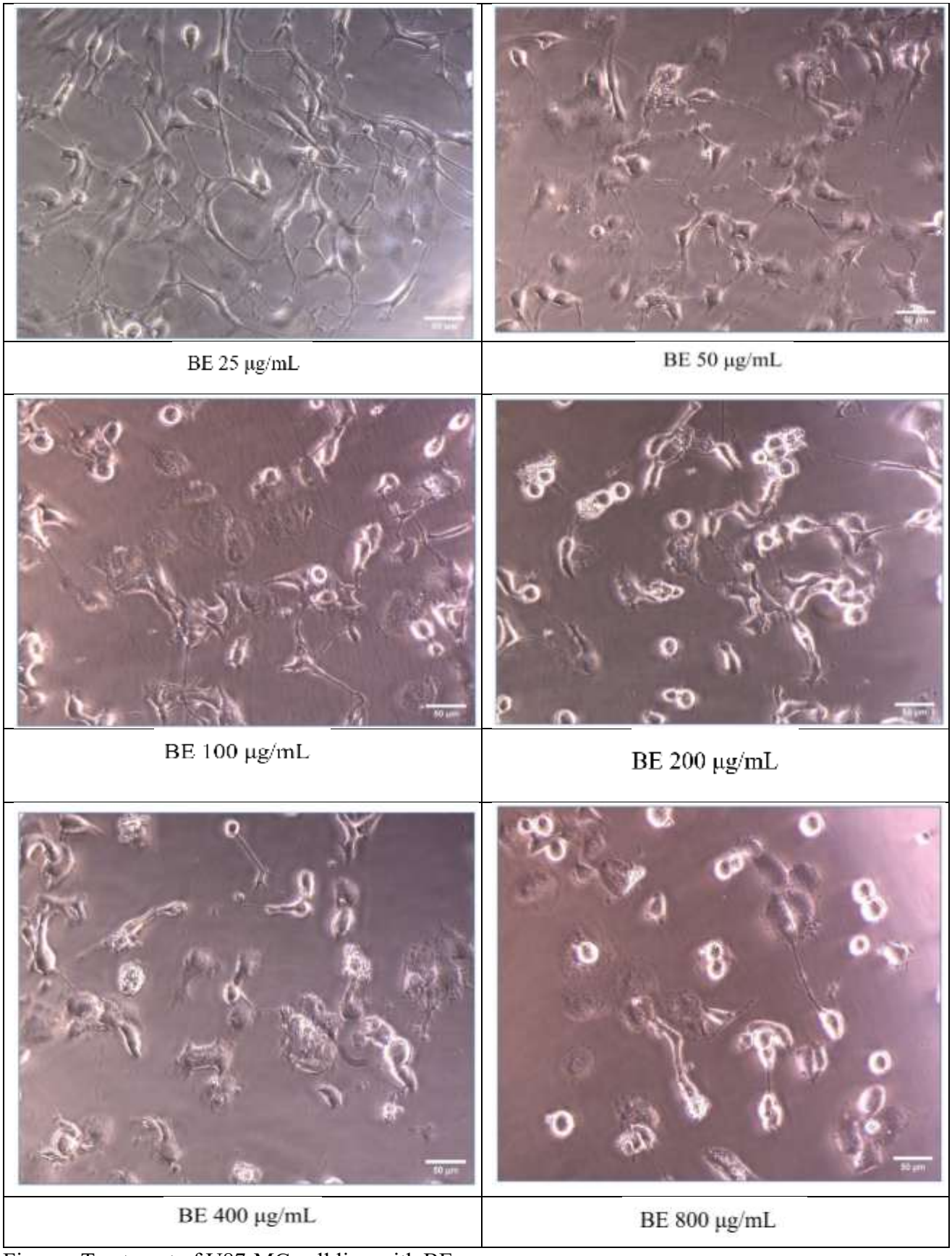

Figure : Treatment of U87-MG cell line with BE

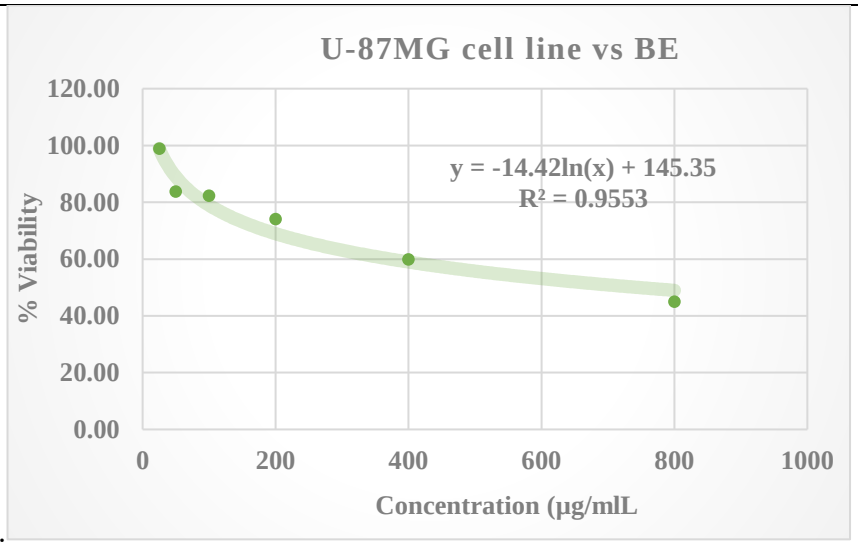

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Figure: Represents R^2 value-Plot between % viability and concentration of U 87-MG cell lines vs *B. crispa* ethanolic extract

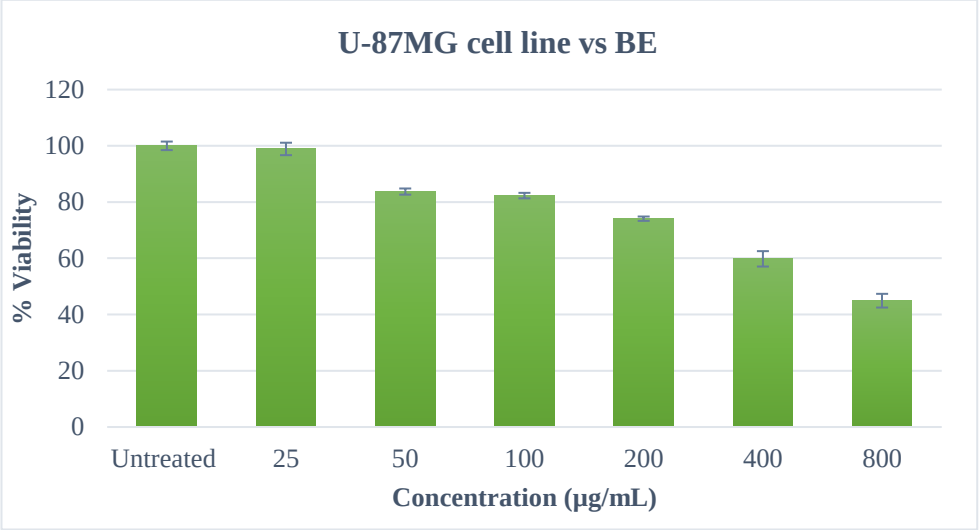

Figure: RepresentsPlot between % viability and concentration of U 87-MG cell lines vs *B. crispa* ethanolic extract

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Table 1. MTT data analysis: A-431 cell line with standard drug (Docetaxel)

	Test concentration µg/mL							
	Blank	**Untreated**	**25**	**50**	**100**	**200**	**400**	**800**
Reading 1	0.01	0.825	0.624	0.567	0.349	0.101	0.064	0.043
Reading 2	0.005	0.843	0.599	0.517	0.341	0.116	0.055	0.069
Reading 3	0.007	0.807	0.635	0.582	0.378	0.111	0.036	0.047
Mean OD	0.007	0.825	0.619	0.555	0.356	0.109	0.052	0.053
Mean OD-Mean Blank		0.8177	0.6120	0.5480	0.3487	0.1020	0.0443	0.0457
Standard deviation		0.0180	0.0184	0.0340	0.0195	0.0076	0.0143	0.0140
Standard error		0.0104	0.0107	0.0196	0.0112	0.0044	0.0083	0.0081
% Standard error		1.2710	1.3026	2.4031	1.3746	0.5393	1.0093	0.9885
% Viability		100	74.85	67.02	42.64	12.47	5.42	5.58
IC_{50}= 53.03 µg/mL								

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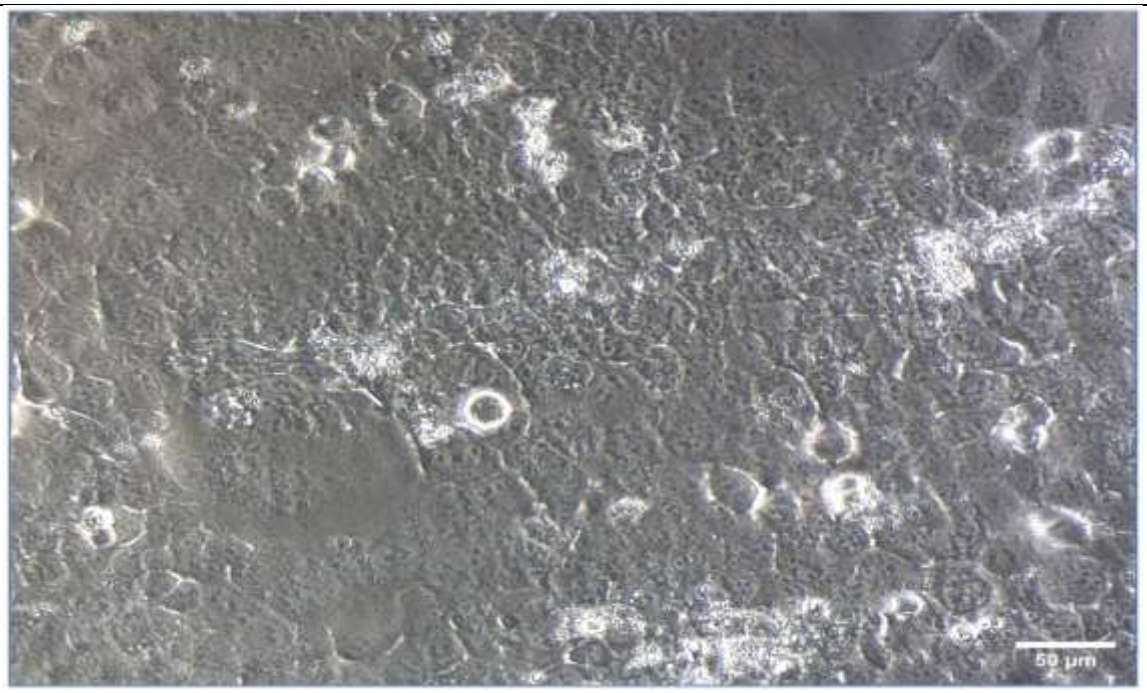

A-431
Untreated

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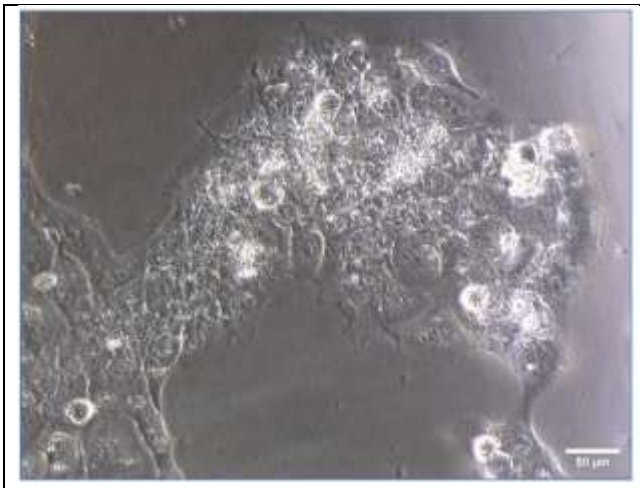
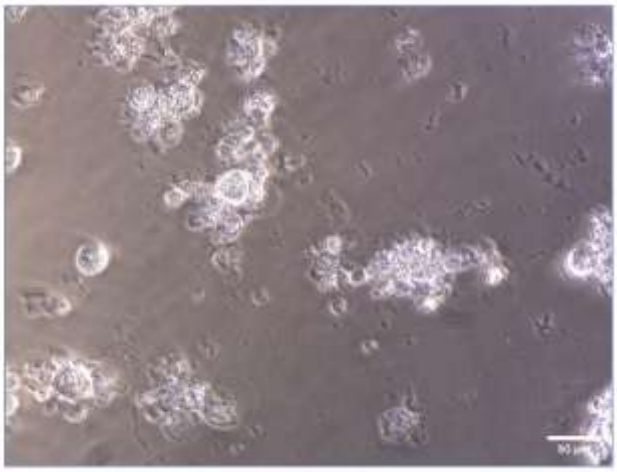


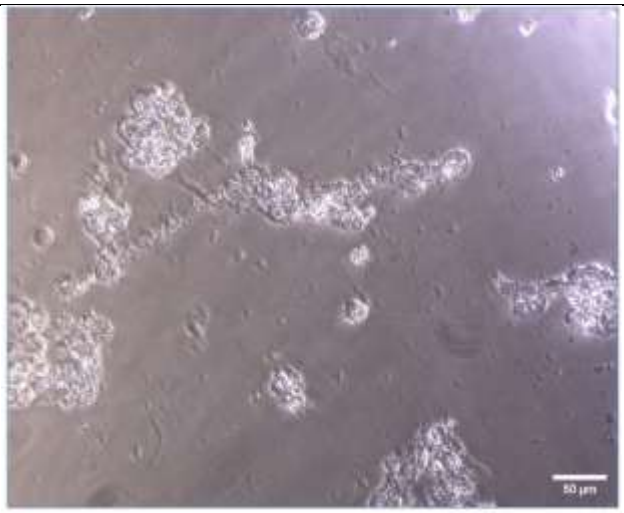
Docetaxel 25 µg/mL

Docetaxel 50 µg/mL

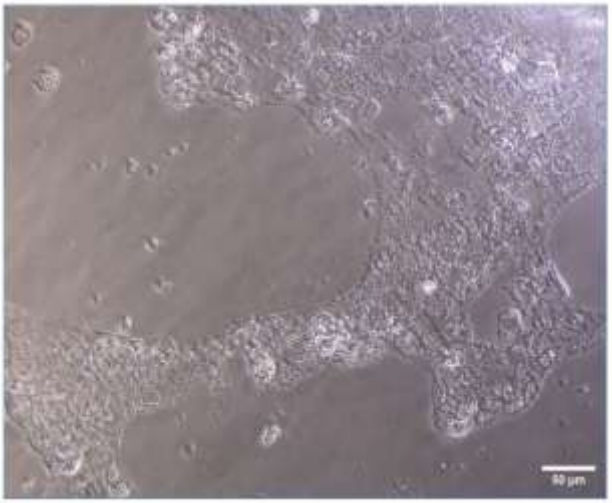


Docetaxel 100 µg/mL

Docetaxel 200 µg/mL



Docetaxel 400 µg/mL



Docetaxel 800 µg/mL

Figure : Treatment of A-431 cell line with standard drug (Docetaxel)

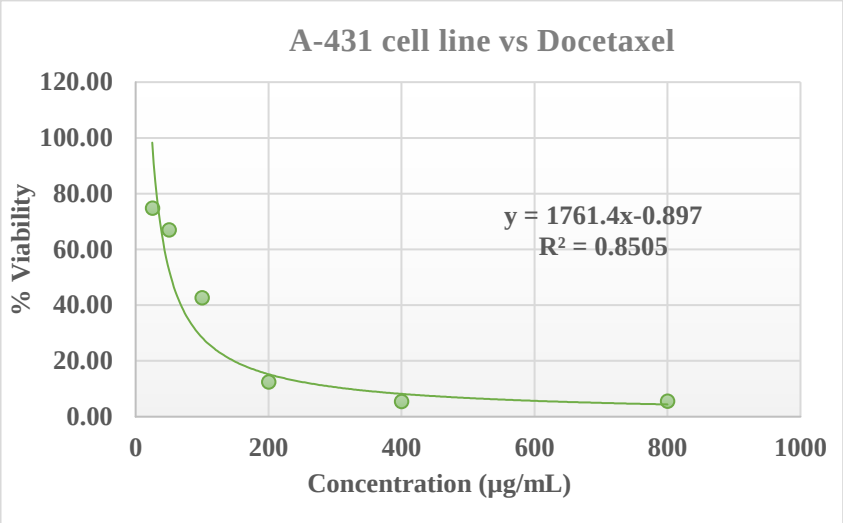


Figure: Represents R^2 value-Plot between % viability and concentration of A-431 cell lines vs Docetaxel

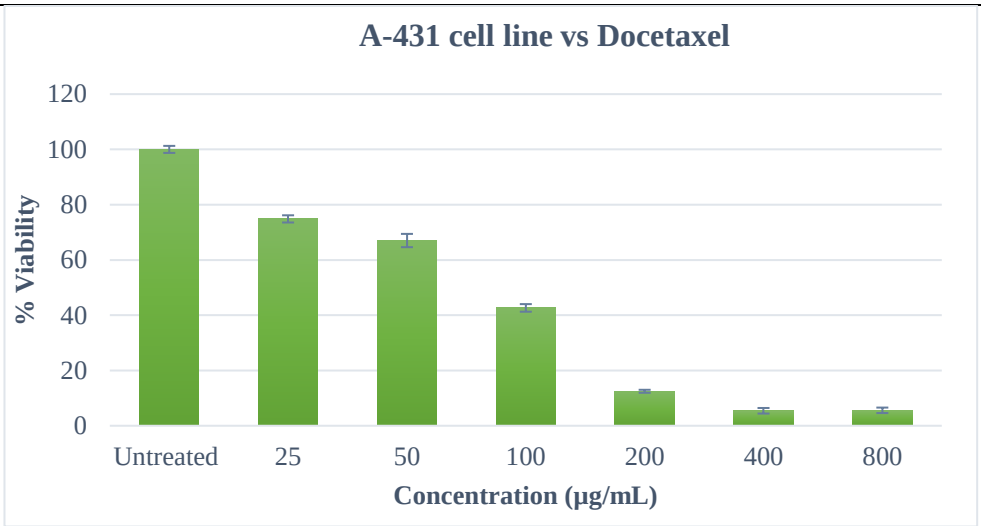


Figure: Represents Plot between % viability and concentration of A-431 cell lines vs Docetaxel

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Table 1. MTT data analysis: A431 cell line vs BE

	Test concentration µg/mL							
	Blank	**Untreated**	**25**	**50**	**100**	**200**	**400**	**800**
Reading 1	0.01	0.825	0.761	0.697	0.627	0.576	0.501	0.383
Reading 2	0.005	0.843	0.768	0.718	0.675	0.499	0.515	0.426
Reading 3	0.007	0.807	0.728	0.704	0.634	0.547	0.479	0.385
Mean OD	0.007	0.825	0.752	0.706	0.645	0.541	0.498	0.398
Mean OD-Mean Blank		0.8177	0.7450	0.6990	0.6380	0.5333	0.4910	0.3907
Standard deviation		0.0180	0.0214	0.0107	0.0259	0.0389	0.0181	0.0243
Standard error		0.0104	0.0123	0.0062	0.0150	0.0225	0.0105	0.0140
% Standard error		1.2710	1.5084	0.7550	1.8309	2.7459	1.2814	1.7136
% Viability		100	91.11	85.49	78.03	65.23	60.05	47.78
IC_{50}= 766.675 µg/mL								

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A-431
Untreated

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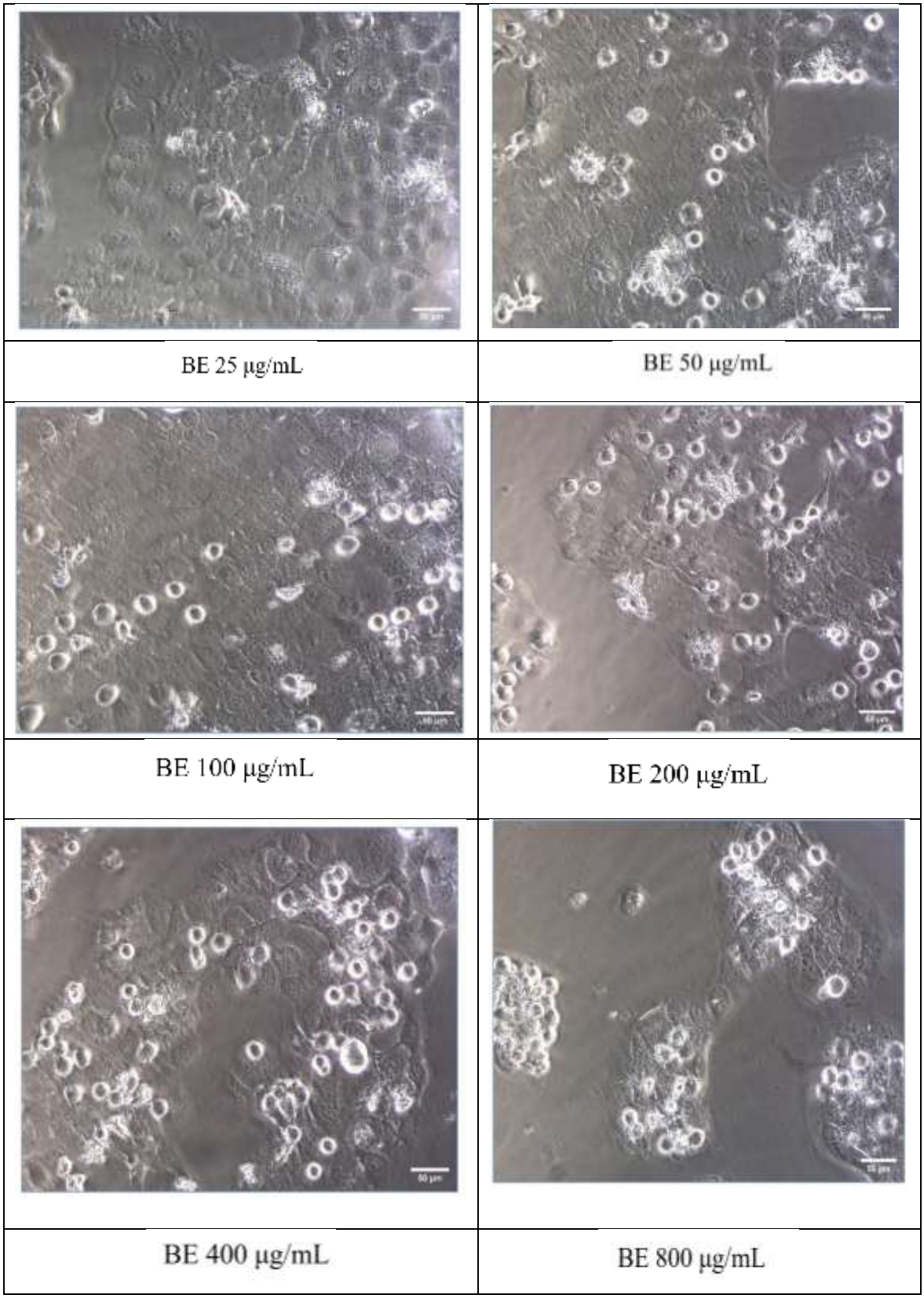

Figure : Treatment of A-431 cell line with BE

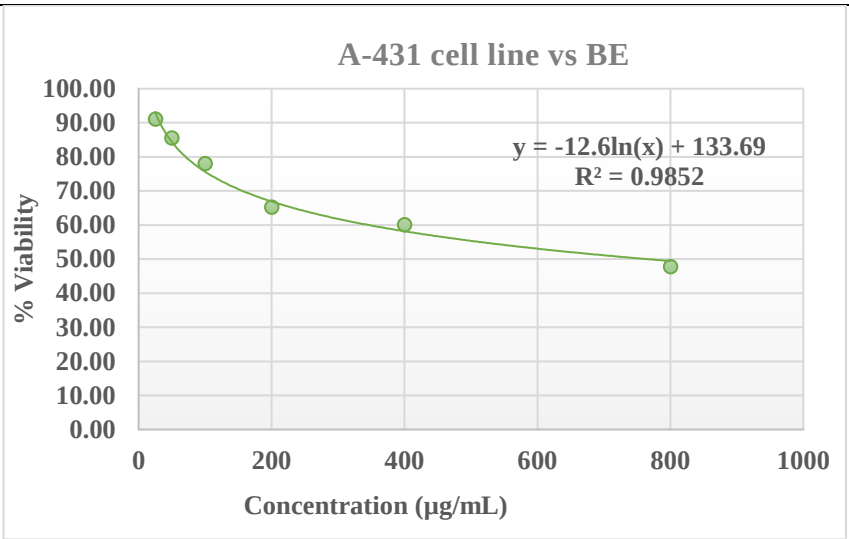

Figure: Represents R^2 value-Plot between % viability and concentration of A-431 cell lines vs *B. crispa* ethanolic extract

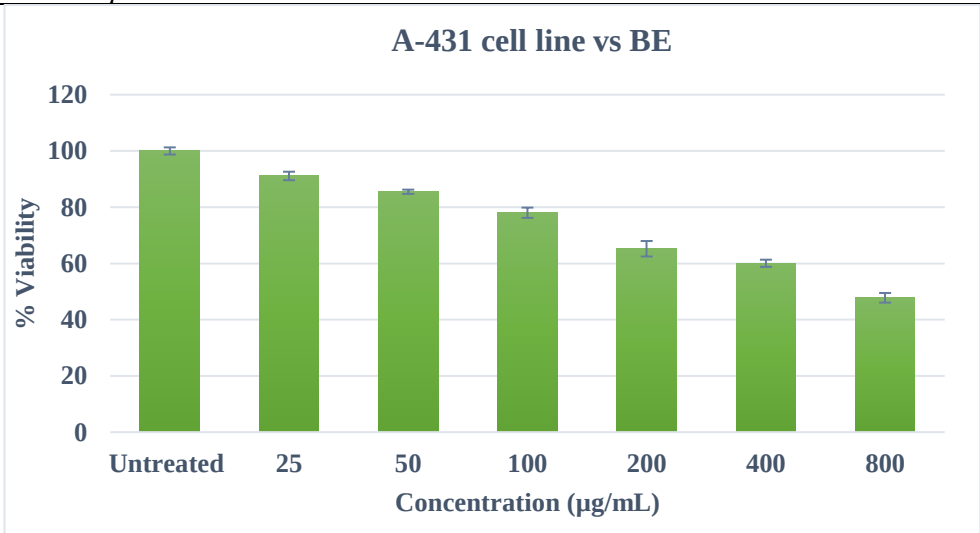

Figure: Represents Plot between % viability and concentration of A-431 cell lines vs *B. crispa* ethanolic extract

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Table 1. MTT data analysis: A431 cell line vs B22-B24W

	Test concentration µg/mL							
	Blank	**Untreated**	**15.625**	**31.25**	**62.5**	**125**	**250**	**500**
Reading 1	0.009	0.747	0.781	0.791	0.759	0.722	0.708	0.3
Reading 2	0.01	0.782	0.758	0.748	0.741	0.721	0.695	0.281
Reading 3	0.007	0.721	0.712	0.711	0.735	0.704	0.677	0.351
Mean OD	0.009	0.750	0.750	0.750	0.745	0.716	0.693	0.311
Mean OD-Mean Blank		0.7413	0.7417	0.7413	0.7363	0.7070	0.6847	0.3020
Standard deviation		0.0306	0.0351	0.0400	0.0125	0.0101	0.0156	0.0362
Standard error		0.0177	0.0203	0.0231	0.0072	0.0058	0.0090	0.0209
% Standard error		2.3839	2.7362	3.1181	0.9727	0.7878	1.2124	2.8191
% Viability		100	100.04	100.00	99.33	95.37	92.36	40.74
IC_{50}= 485.04 µg/mL								

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A-431
Untreated

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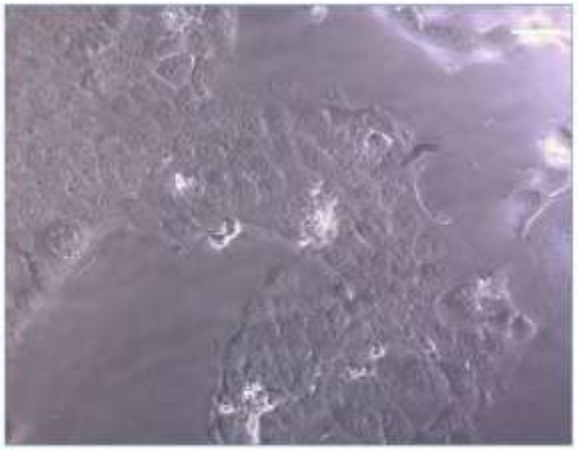
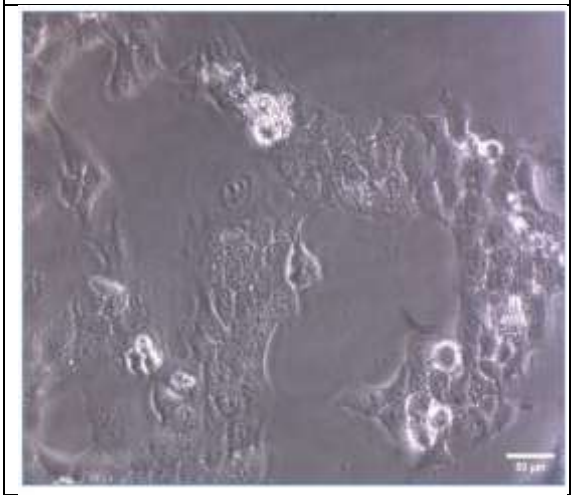
B22-B24W
15.625 µg/mL

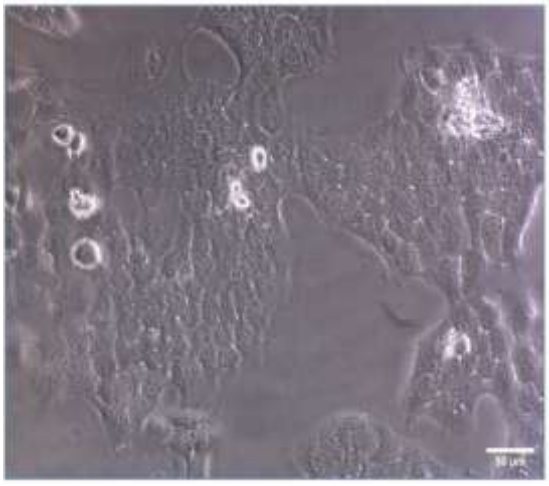


B22-B24W
31.25 µg/mL



B22-B24W
62.5 µg/mL

B22-B24W
125 µg/mL



B22-B24W
250 µg/mL

B22-B24W
500 µg/mL

Figure : Treatment of A-431 cell line with B22-B24W

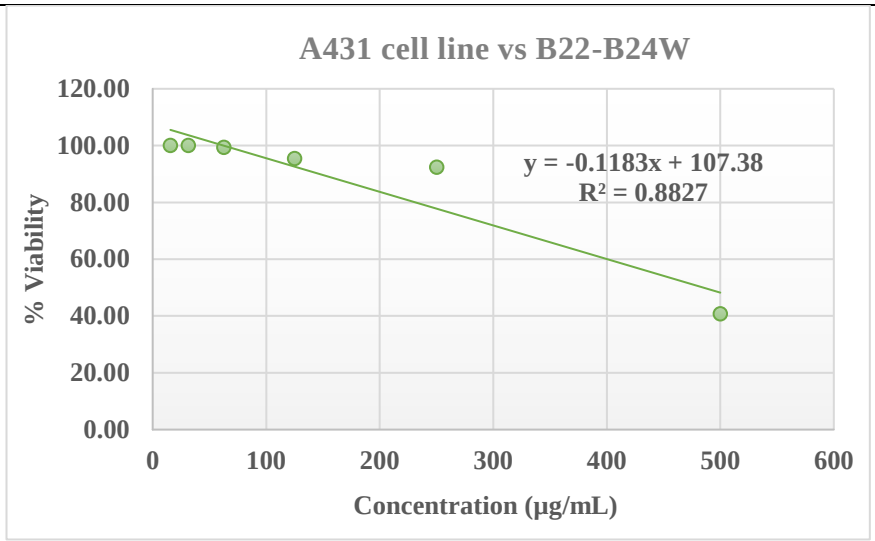


Figure: Represents R^2 value-Plot between % viability and concentration of A-431 cell lines vs isolated fraction (B22-B24W)

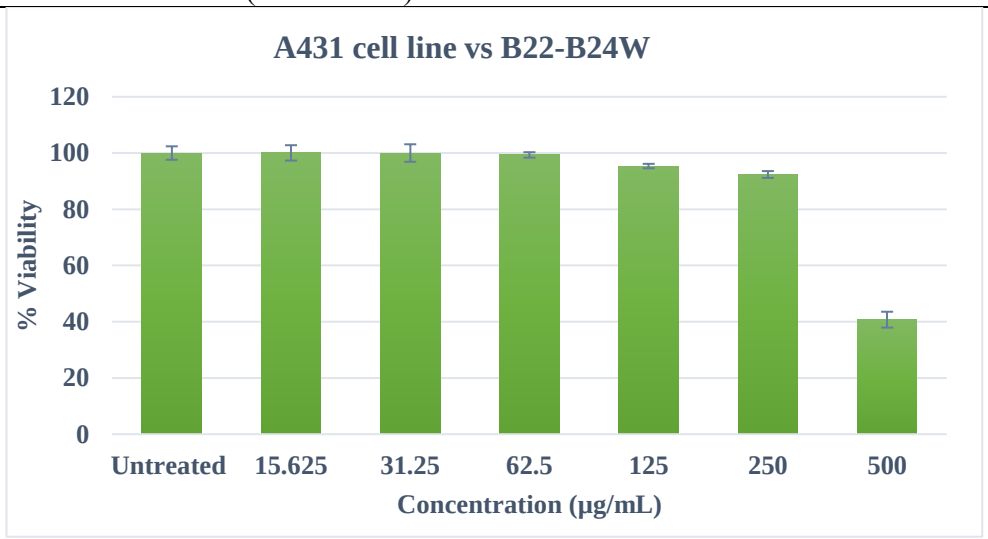


Figure: Represents Plot between % viability and concentration of A-431 cell lines vs isolated fraction (B22-B24W)

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AO/PI staining

Fluorescence microscopy via acridine orange and propidium iodide was also conducted to determine viable cell count and the fluorescent micrographs obtained are represented in Figure 5B(d). The isolated fraction (B22-B24) significantly (P<0.05) inhibited the proliferation of A-431 in a dose-dependent manner.

Table 1. MTT data analysis: A-431 cell line with B22-B24W

Sample	Dead	Live	Total	%Live	Avg	%Dead	Avg.	p-value (T-test)
Untreated	10	607	617	98.4	98.0	1.6	2.0	
	16	667	683	97.7		2.3		
	12	623	635	98.1		1.9		
	12	583	595	98.0		2.0		
Standard - 53.03 µg/mL	93	281	374	75.1	74.2	24.9	25.8	0.000022
	107	280	387	72.4		27.6		
	88	265	353	75.1		24.9		
	88	253	341	74.2		25.8		
B-22-24W- 485.04 µg/mL	34	301	335	89.9	89.1	10.1	10.9	0.001113
	51	364	415	87.7		12.3		
	44	324	368	88.0		12.0		
	34	342	376	91.0		9.0		

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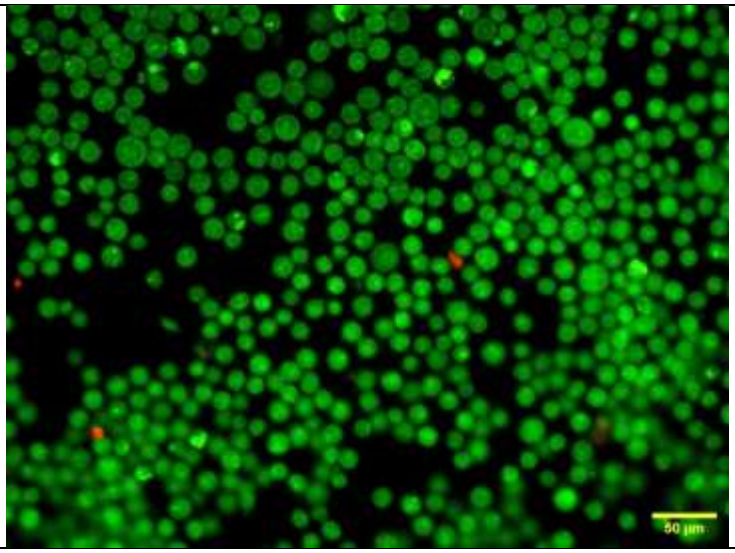
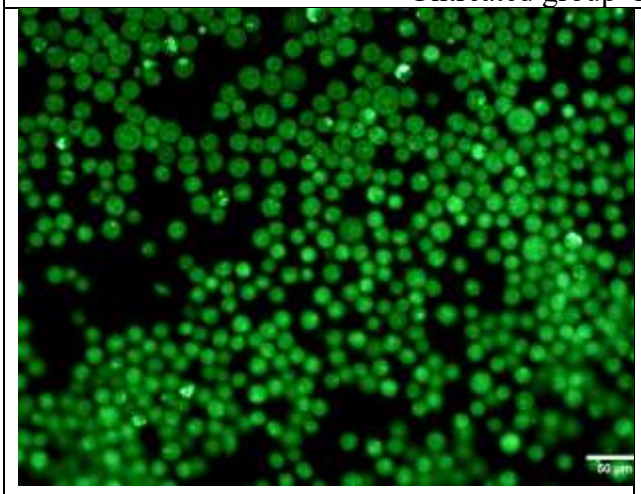
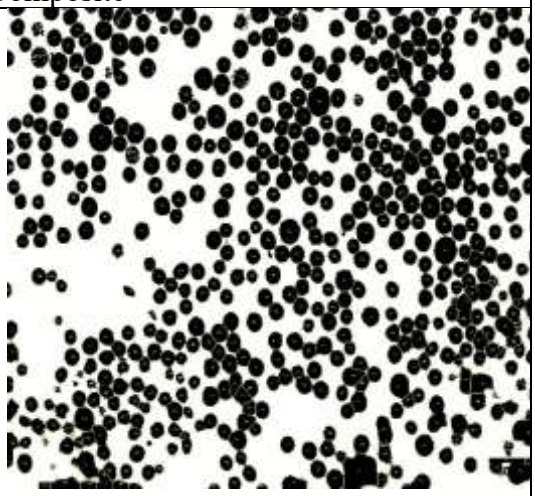
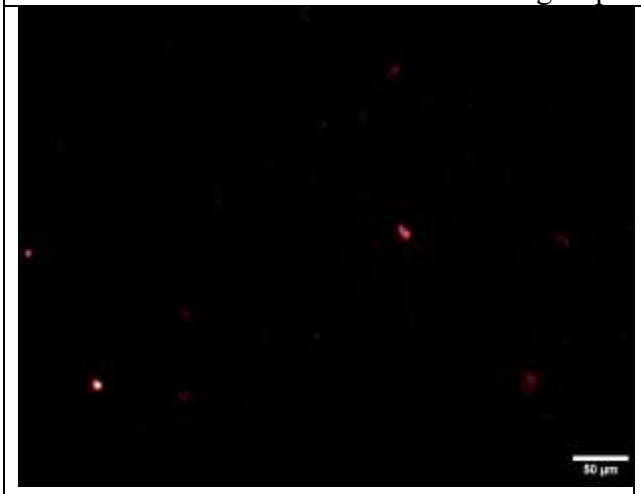


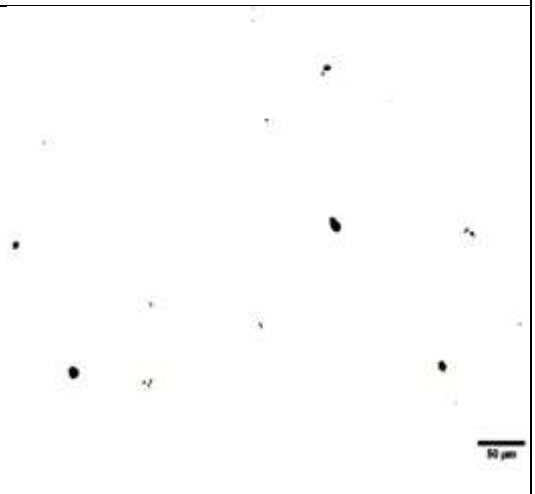
Untreated group-Composite



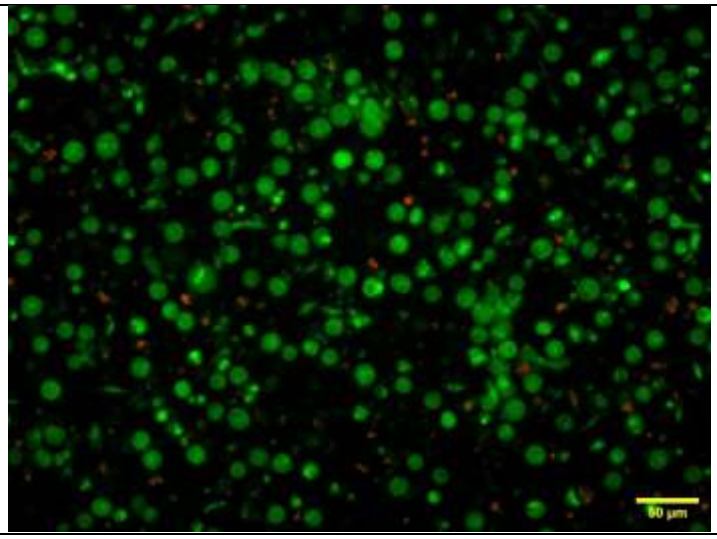


Untreated group-Viable cells

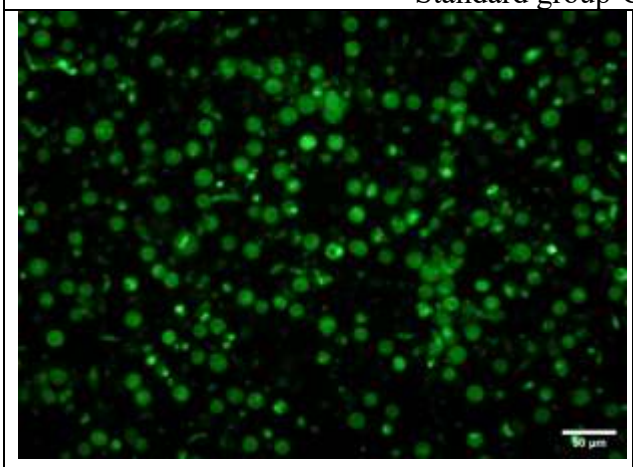


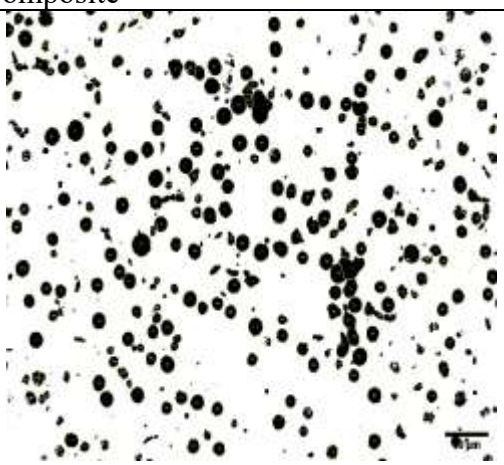


Untreated group-Necrotic cells

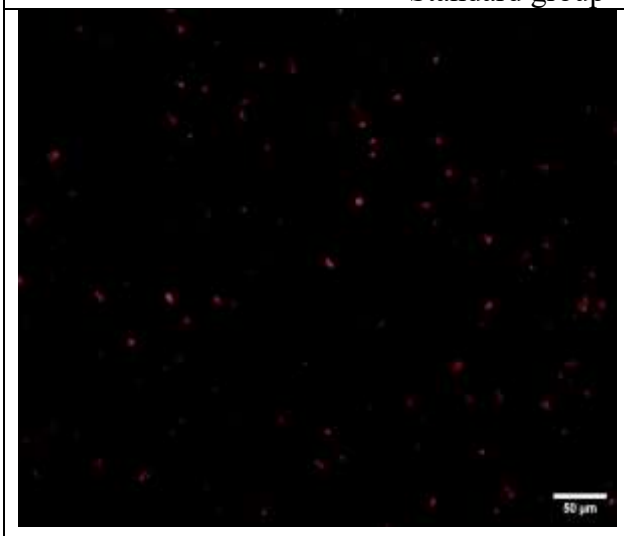


Standard group-Composite





Standard group-Viable cells



Standard group-Necrotic cells

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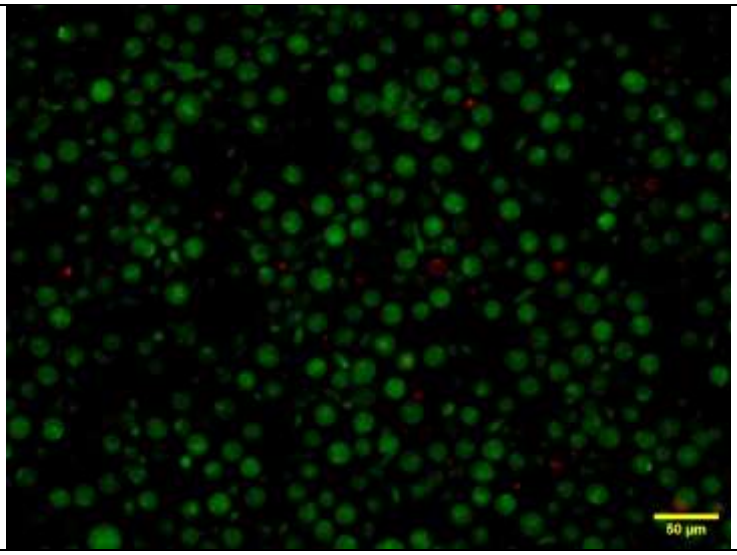
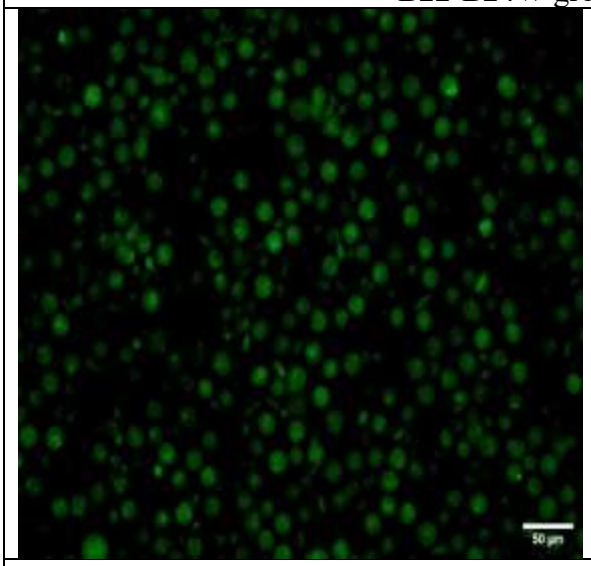
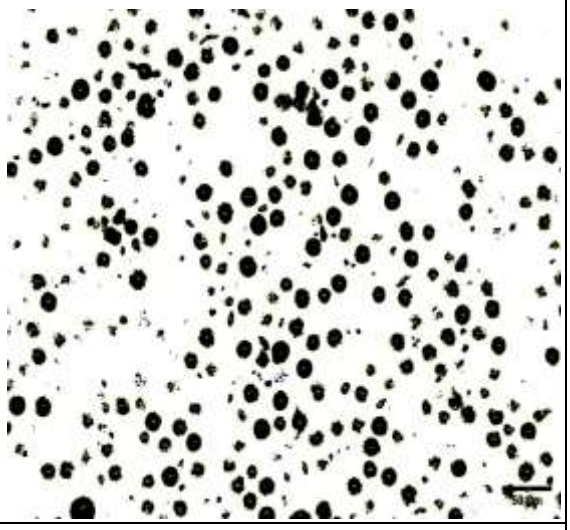
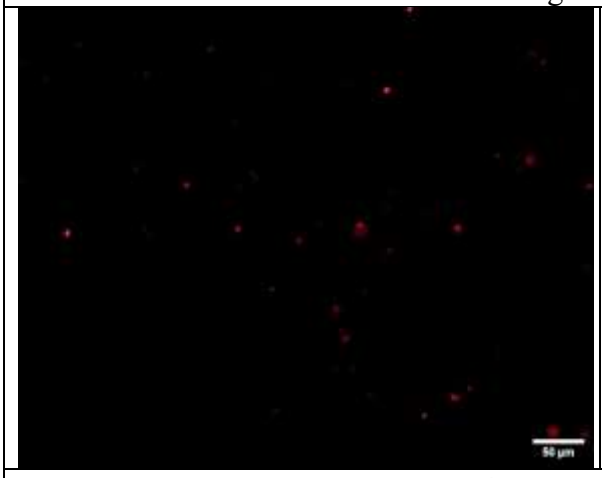


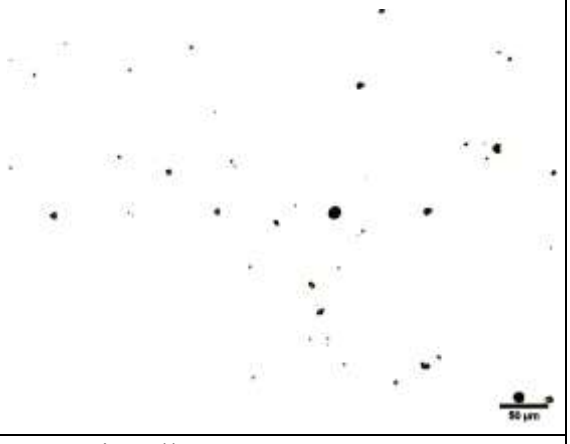
B22-B24W group-Composite





B22-B24W group-Viable cells

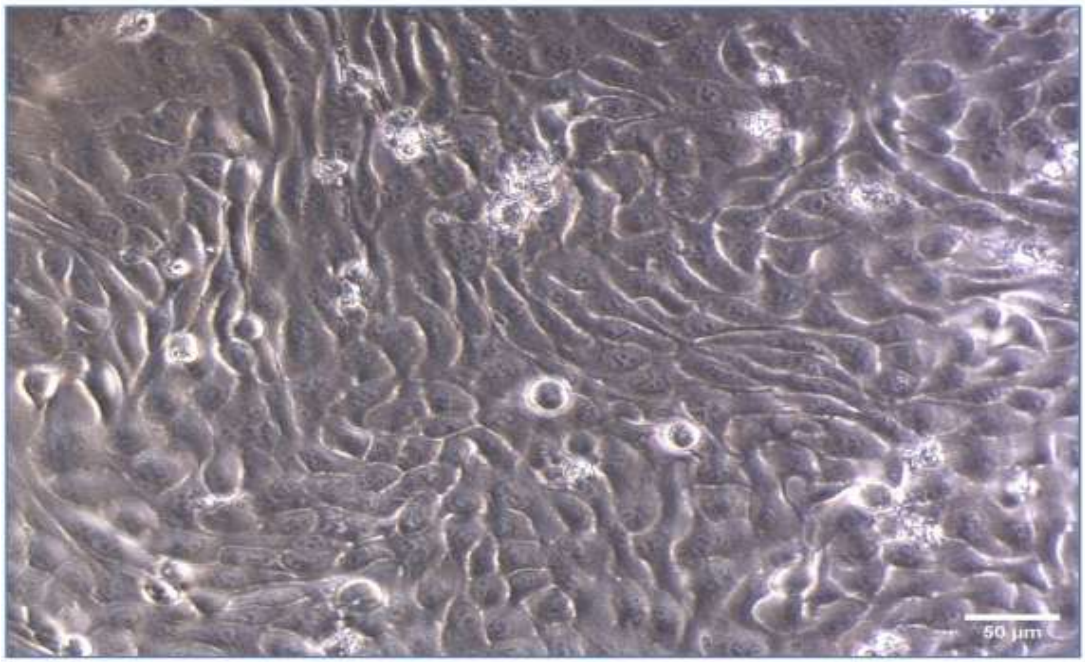




B22-B24W group-Necrotic cells

Table 1. MTT data analysis: HaCaT cell line with Docetaxel

	Test concentration µg/mL							
	Blank	**Untreated**	**25**	**50**	**100**	**200**	**400**	**800**
Reading 1	0.007	0.828	0.326	0.195	0.084	0.024	0.029	0.02
Reading 2	0.008	0.849	0.369	0.207	0.083	0.035	0.039	0.059
Reading 3	0.004	0.892	0.332	0.186	0.048	0.053	0.024	0.039
Mean OD	0.006	0.856	0.342	0.196	0.072	0.037	0.031	0.039
Mean OD-Mean Blank		0.8500	0.3360	0.1897	0.0653	0.0310	0.0243	0.0330
Standard deviation		0.0326	0.0233	0.0105	0.0205	0.0146	0.0076	0.0195
Standard error		0.0188	0.0134	0.0061	0.0118	0.0085	0.0044	0.0113
% Standard error		2.2159	1.5818	0.7156	1.3926	0.9944	0.5188	1.3247
% Viability		100	39.53	22.31	7.69	3.65	2.86	3.88
IC_{50}= 12.93 µg/mL								



HaCaT
Untreated

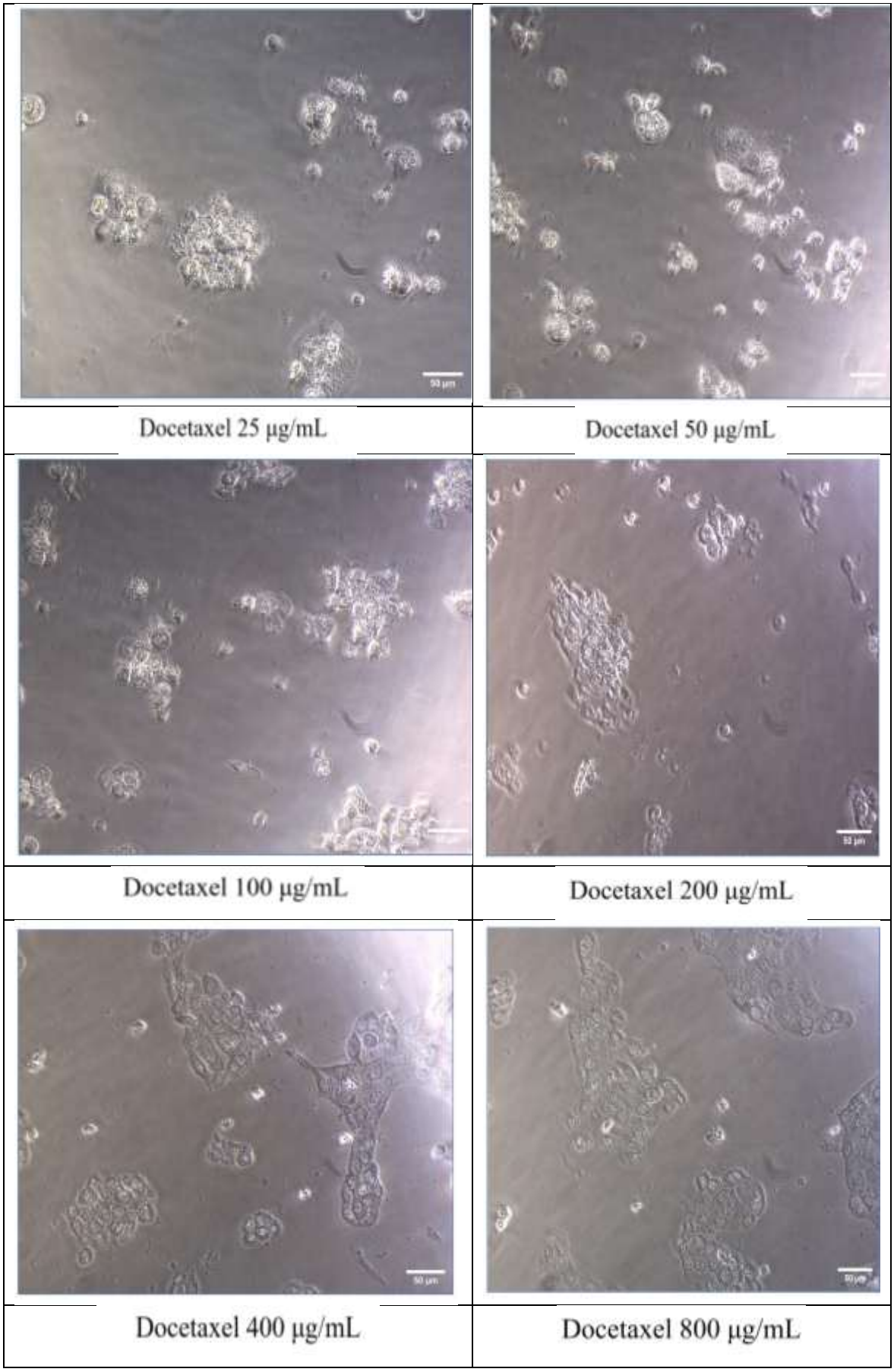


Figure : Treatment of HaCaT cell line with Docetaxel

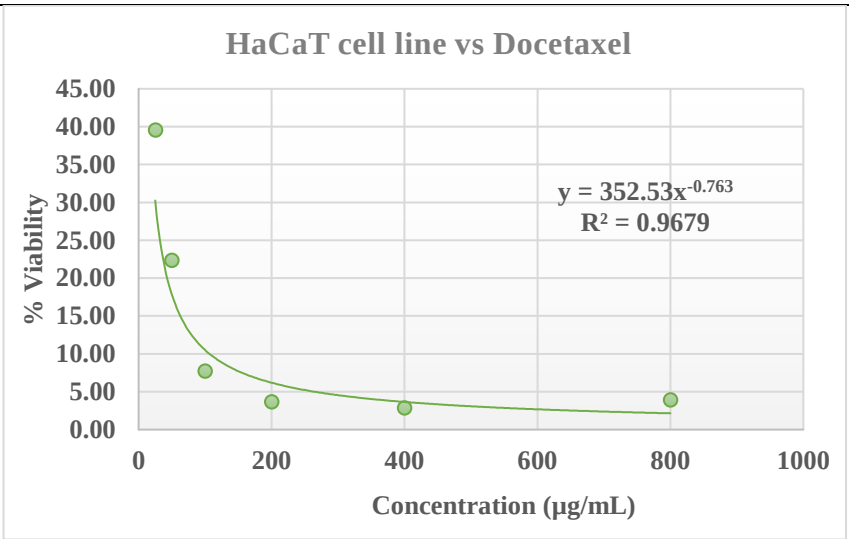


Figure: Represents R^2 value-Plot between % viability and concentration of HaCaT cell lines vs Docetaxel

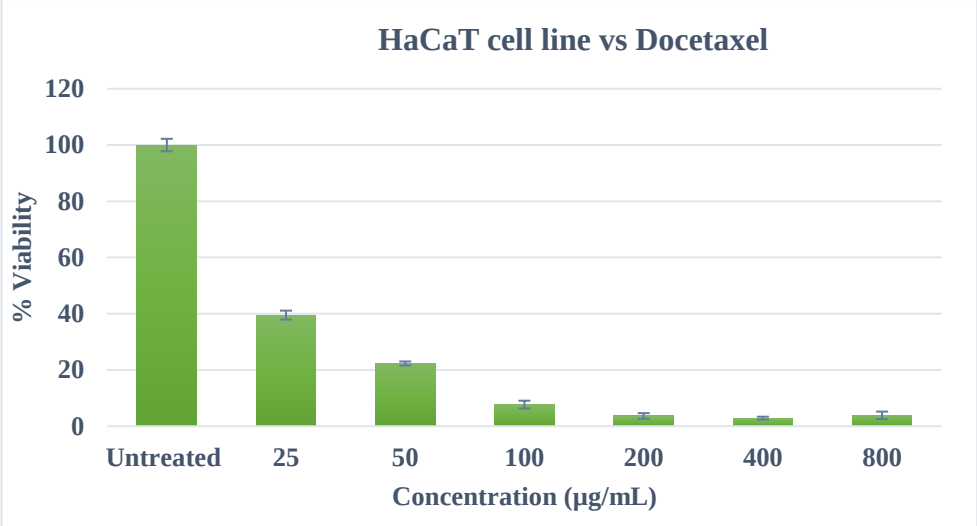


Figure: Represents plot between % viability and concentration of HaCaT cell lines vs Docetaxel

Table 1. MTT data analysis: HaCaT cell line vs BE

	Test concentration µg/mL							
	Blank	**Untreated**	**25**	**50**	**100**	**200**	**400**	**800**
Reading 1	0.007	0.828	0.845	0.839	0.847	0.805	0.841	0.846
Reading 2	0.008	0.849	0.882	0.837	0.867	0.845	0.871	0.815
Reading 3	0.004	0.892	0.864	0.847	0.888	0.868	0.815	0.85
Mean OD	0.006	0.856	0.864	0.841	0.867	0.839	0.842	0.837
Mean OD-Mean Blank		0.8500	0.8573	0.8347	0.8610	0.8330	0.8360	0.8307
Standard deviation		0.0326	0.0185	0.0053	0.0205	0.0319	0.0280	0.0192
Standard error		0.0188	0.0107	0.0031	0.0118	0.0184	0.0162	0.0111
% Standard error		2.2159	1.2567	0.3594	1.3926	2.1654	1.9035	1.3012
% Viability		100	100.86	98.20	101.29	98.00	98.35	97.73
IC_{50}= Not predicted								

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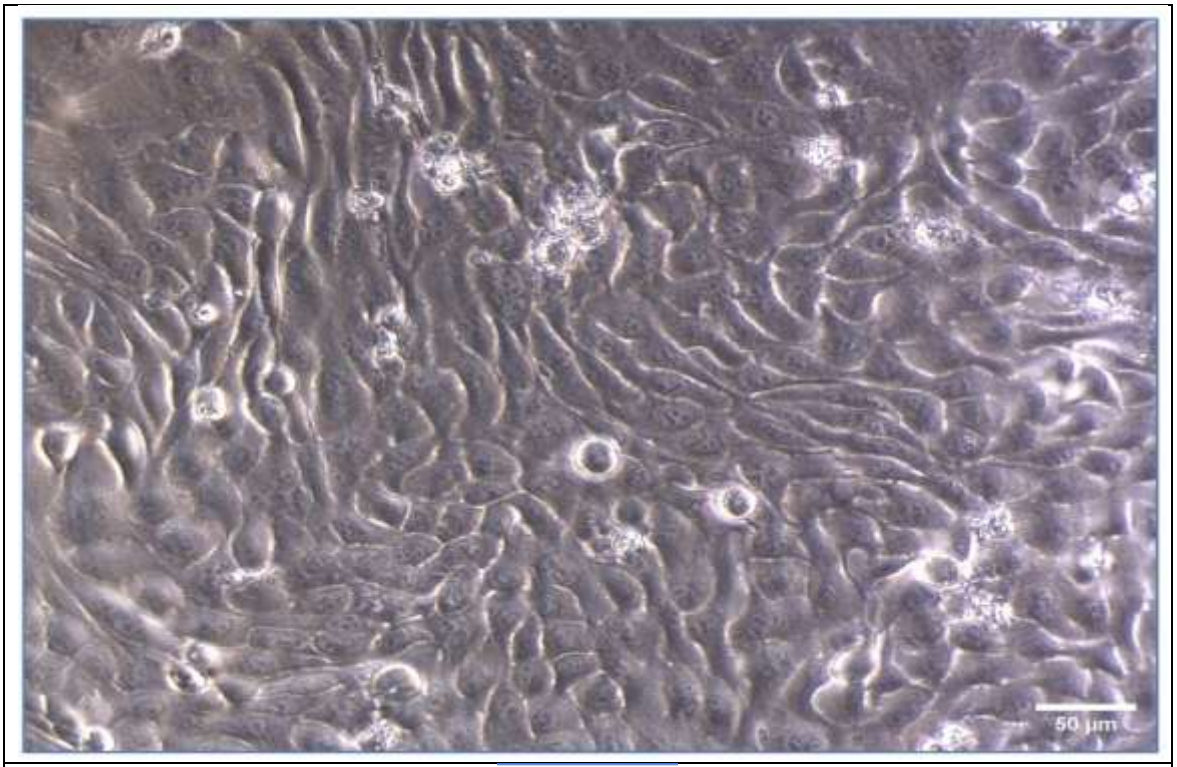

IIaCaT
Untreated

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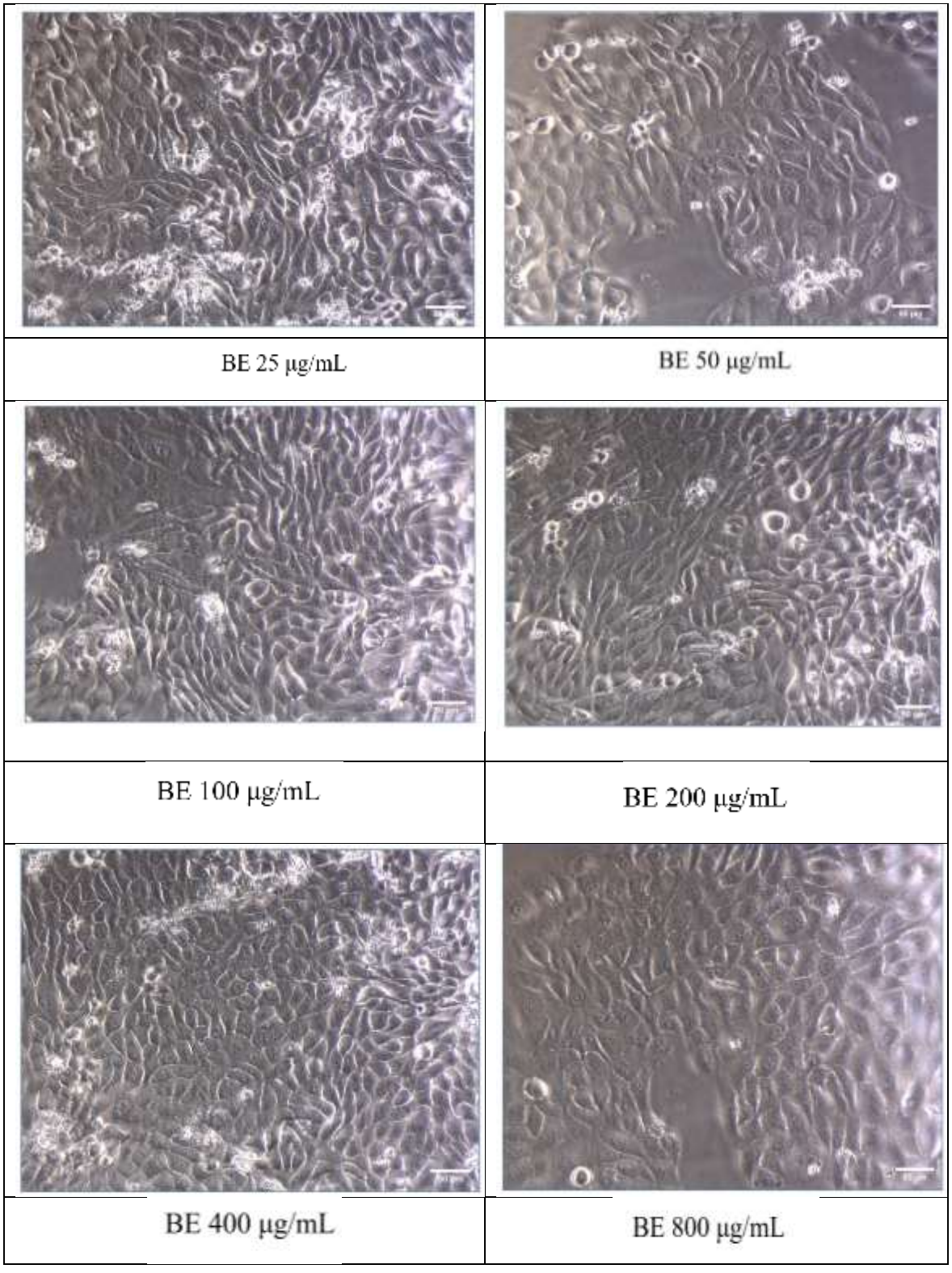

Figure : Treatment of HaCaT cell line with BE

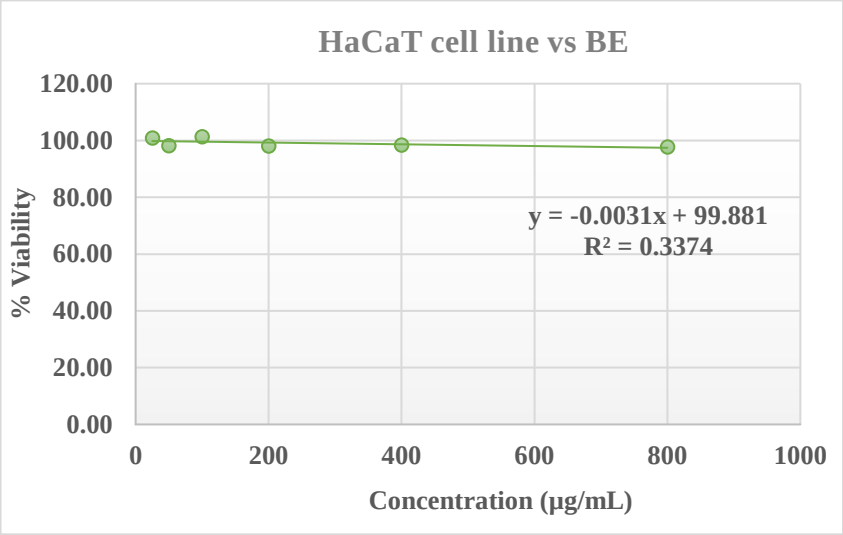

Figure: Represents R^2 value-Plot between % viability and concentration of HaCaT cell lines vs *B. crispa* ethanolic extract

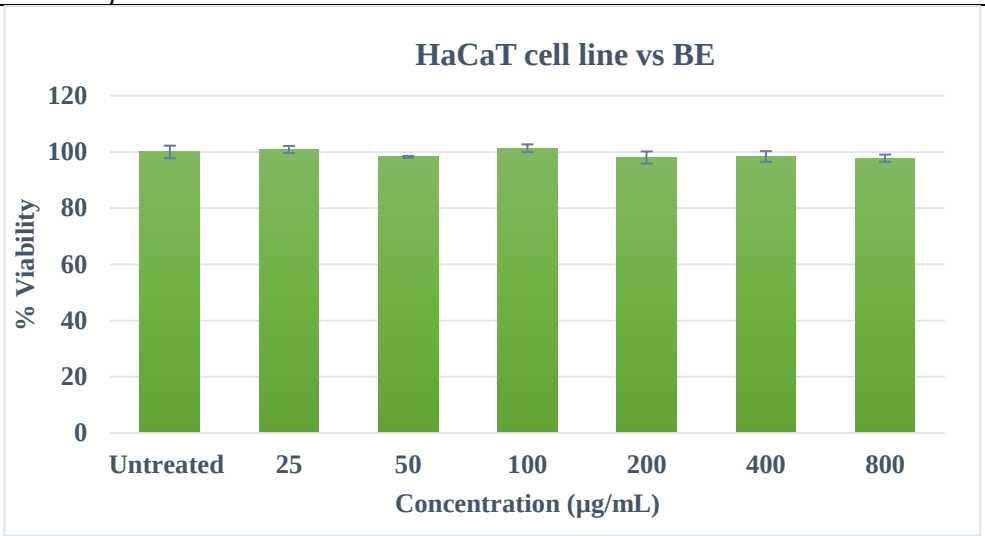

Figure: Represents plot between % viability and concentration of HaCaT cell lines vs Docetaxel

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14.20 Statistical analysis of purified fraction (B22-B24W)

One-way ANOVA, a statistical technique employed to compare the means of multiple groups and identify any significant differences among them, was used to calculate the p value. Significant differences were observed in the means of cell viability across test concentrations ($F = 333.413$, $p < 0.001$). The **untreated s**ample exhibited a high mean absorbance (0.750±0.031), indicative of normal cell viability. The absorbance remained comparable to that of the untreated sample at lower concentrations (15.625 to 62.5), reflecting minimal cytotoxic effects. However, cell viability steadily decreased with increasing concentrations, reaching a low mean value of 0.311±0.036 at the highest tested concentration (500 µg/ml) (Table 1).

Table 1 Difference between test concentrations and drug concentration (µg/mL)

B22-B24	Mean	Std. Deviation	F value	p value
Blank	0.009	0.002	333.413	0.000
Untreated	0.750	0.031		
15.625	0.750	0.035		
31.25	0.750	0.04		
62.5	0.745	0.012		
125	0.716	0.01		
250	0.693	0.016		
500	0.311	0.036		

Tukey's post-hoc analysis revealed significant differences in cell viability among the concentrations, indicating a dose-dependent cytotoxic effect. The blank group consistently showed the lowest absorbance, which was significantly different from all other groups ($p < 0.001$). Lower concentrations (15.625, 31.25, and 62.5 µg/mL) exhibited negligible differences when compared to the untreated group, forming a homogeneous subset

characterized by high cell viability and minimal cytotoxicity. Intermediate concentrations (125 and 250 µg/mL) caused slight but statistically insignificant reductions in viability, indicating gradual cytotoxicity. In contrast, the highest concentration (500 µg/mL) yielded the lowest mean viability, significantly differing from all other groups ($p < 0.001$), thereby confirming its pronounced cytotoxicity and forming a distinct subset. This analysis highlights a clear dose-dependent reduction in cell viability at higher concentrations (Table 2).

Table 2 Multiple Comparisons using Tukey

(I) Test concentration	(J) Test concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Blank	Untreated	-.741333	0.022	0.000	-0.816	-0.667
	15.625	-.741667	0.022	0.000	-0.816	-0.667
	31.25	-.741333	0.022	0.000	-0.816	-0.667
	62.5	-.736333	0.022	0.000	-0.811	-0.662
	125	-.707000	0.022	0.000	-0.782	-0.632
	250	-.684667	0.022	0.000	-0.759	-0.610
	500	-.302000	0.022	0.000	-0.377	-0.227
Untreated	Blank	.741333	0.022	0.000	0.667	0.816
	15.625	-0.000333	0.022	1.000	-0.075	0.074
	31.25	0.000000	0.022	1.000	-0.075	0.075
	62.5	0.005000	0.022	1.000	-0.070	0.080
	125	0.034333	0.022	0.749	-0.040	0.109
	250	0.056667	0.022	0.216	-0.018	0.131
	500	.439333	0.022	0.000	0.365	0.514
15.625	Blank	.741667	0.022	0.000	0.667	0.816
	Untreated	0.000333	0.022	1.000	-0.074	0.075
	31.25	0.000333	0.022	1.000	-0.074	0.075
	62.5	0.005333	0.022	1.000	-0.069	0.080
	125	0.034667	0.022	0.741	-0.040	0.109
	250	0.057000	0.022	0.211	-0.018	0.132
	500	.439667	0.022	0.000	0.365	0.514
31.25	Blank	.741333	0.022	0.000	0.667	0.816

(I) Test concentration	(J) Test concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	Untreated	0.000000	0.022	1.000	-0.075	0.075
	15.625	-0.000333	0.022	1.000	-0.075	0.074
	62.5	0.005000	0.022	1.000	-0.070	0.080
	125	0.034333	0.022	0.749	-0.040	0.109
	250	0.056667	0.022	0.216	-0.018	0.131
	500	.439333	0.022	0.000	0.365	0.514
62.5	Blank	.736333	0.022	0.000	0.662	0.811
	Untreated	-0.005000	0.022	1.000	-0.080	0.070
	15.625	-0.005333	0.022	1.000	-0.080	0.069
	31.25	-0.005000	0.022	1.000	-0.080	0.070
	125	0.029333	0.022	0.862	-0.045	0.104
	250	0.051667	0.022	0.307	-0.023	0.126
	500	.434333	0.022	0.000	0.360	0.509
125	Blank	.707000	0.022	0.000	0.632	0.782
	Untreated	-0.034333	0.022	0.749	-0.109	0.040
	15.625	-0.034667	0.022	0.741	-0.109	0.040
	31.25	-0.034333	0.022	0.749	-0.109	0.040
	62.5	-0.029333	0.022	0.862	-0.104	0.045
	250	0.022333	0.022	0.962	-0.052	0.097
	500	.405000	0.022	0.000	0.330	0.480
250	Blank	.684667	0.022	0.000	0.610	0.759
	Untreated	-0.056667	0.022	0.216	-0.131	0.018
	15.625	-0.057000	0.022	0.211	-0.132	0.018
	31.25	-0.056667	0.022	0.216	-0.131	0.018
	62.5	-0.051667	0.022	0.307	-0.126	0.023
	125	-0.022333	0.022	0.962	-0.097	0.052
	500	.382667	0.022	0.000	0.308	0.457
500	Blank	.302000	0.022	0.000	0.227	0.377
	Untreated	-.439333	0.022	0.000	-0.514	-0.365
	15.625	-.439667	0.022	0.000	-0.514	-0.365
	31.25	-.439333	0.022	0.000	-0.514	-0.365
	62.5	-.434333	0.022	0.000	-0.509	-0.360
	125	-.405000	0.022	0.000	-0.480	-0.330
	250	-.382667	0.022	0.000	-0.457	-0.308

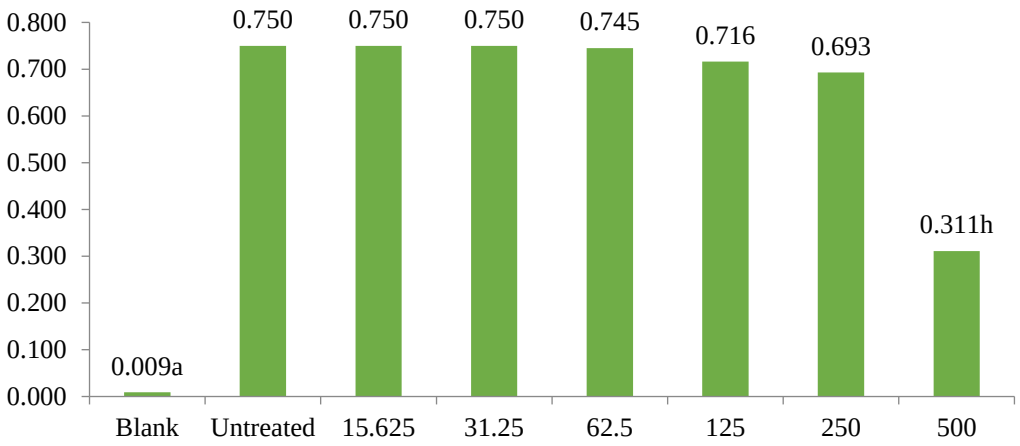

Figure 1 Differences in cell viability across concentrations