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In-vitro Anti-Cancer Activity of Extracts *Dracaen Cinnabari Balf. F* Resin from Socotra Island in Yemen Republic

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Abstract

Plants have a long history of use in treatment of cancer, Since many years, plants were known to possess anticancer activities against different cancer cell lines. In this paper, we report a study based on anticancer properties of Dragon *cinnabari* resin. The resin of plant material was collected, shade dried and extracted with different solvents using soxhlet extraction procedure. *In vitro* anticancer activity is assayed with standard MTT colorimetric procedure against MCF-7 cell line. From the analysis it was found that Ether and Ethyl acetate of dragon *cinnabari* Balf. f showed nearly 50% MCF-7 cell line inhibition at 100 µg/ml tested dose, whereas other extracts did not display much anticancer activities against MCF-7 breast cancer cell line. Based on the cytotoxicity studies against MCF-7 cell lines the ether and ethyl acetate extracts could be used as potential source for anticancer drugs.

Keywords: *Dracaena cinnabari Blaf. F*; Plant extract; Anticancer; MCF-7 cell line

Introduction

The plants can produce many metabolic compounds mainly during the secondary metabolites, plant extracts contain several compounds that have biological active which used as natural medicine [1]. Today herbal derivatives are considered as the basis for a large proportion of the medications in traditional and modern systems of medicine [2]. Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts. Resin is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic, purposes some of the active compounds inhibit the growth of disease causing microbes either singly or in combination [3]. Dragon's blood tree is a non-specific name for dark red resinous exudations from different plant species endemic to various regions around globe that belongs to four genera *Dracaena* spp. (Agavaceae), *Croton* spp. (Euphorbiaceae), *Daemonorops* spp. (Palmaceae) and *Pterocarpus* spp. (Fabaceae) have a long history of being used as a traditional medicine the world over. Medicinal use of dragon's blood dates back to the ancient Greeks, Romans, Chinese and Arabs [4]. However, *Dracaena cinnabari* Balf. f. (*D. cinnabari*) belongs to Agavaceae family, which is commonly known as Damm Al- akhwain in Yemen. It is endemic to the Socotra Island, Yemen. *D. cinnabari* resin has traditionally been used to treat diarrhea, wounds, fevers, ulcers, hemorrhage, control bleeding, fractures, and burns [5]. Plant has anti-microbial and cytotoxicity effect. Some constituents of *Dracaena cinnabari* have been identified: Dracophan, ametacyclophan, Cinnabaron, Abiflavonoids, Numerous phenolic compounds belong to the homoisoflavonoids and chalcons, Sterol, triterpenoids and a new biflavonoids were isolated from this plant. Despite its wide uses, little research has been done to know about its true source, quality control, bioactive compounds and clinical applications. Therefore, it is of great interest to carry out a screening of these plant parts in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents [5-7]. The systematic screening of them may result in the discovery of novel active compounds. *Dracaena cinnabari Balf. F* resin was collected from Socotra Island (Yemen) on May 2014. Thus, in the present study attempts were made to investigate its anticancer activity of the resin extract of dragon cinnabari on MCF-7 cell line by standard MTT colorimetric procedure.

Anticancer Activity

There are many different anticancer herbs that have been used by different cultures throughout time for medicinal purposes, anticancer herbs come in many forms one of which is a type of thistle plants [8-10,11-19] Cancer is considered one of the most common causes of mortality worldwide. Progress made in cancer therapy has not been sufficient to a significantly lower annual death rate from most tumor types, and there is an urgent need for new strategies in cancer control [11]. For centuries, people have been using plants for their therapeutic values. Today 85000 plants have been documented for therapeutic use globally [12]. The World Health Organization (WHO) estimates that almost 75% of World's population has therapeutic experience with herbal drugs. Cancer is one of the most dangerous diseases in humans and presently there is a considerable scientific discovery of new anticancer agents from natural products [13]. The potential of using the natural products as anticancer drugs was recognized in 1950's by U.S. Natural Cancer Institute (NCI) since 1950 major contributions have taken for the discovery of naturally occurring anticancer drugs [14]. *Dracaen cinnabari* Balf. F. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, and infection during confinement, toothache and also it is used by the people in Yemene to cure diarrhea [5,15]. Biological activities such as anti-inflammatory and hepatoprotective activities were reported [16-19]. However, no work has been reported on the anticancer property of this plant. Keeping in view, the present study has been undertaken to investigate anticancer activity of the different extract of *Dracaen cinnabari* Balf. F against MCF-7 cells lines.

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Materials and Methods

Materials

The MCF-7 cell line (from NCCS, Pune), Cells in appropriate medium: DMEM-High glucose (Himedia), Adjustable multichannel pipettes and a pipettor (from Thermo Scientific, USA), MTT Reagent (5 mg/ml) (From Himedia),0.5DMSO (Himedia), D-PBS (Invitrogen), 96-well plate for culturing the cells (From Corning, USA), 96-well ELISA plate reader or spectrophotometer capable of measuring the absorbance (From Biotech), Inverted microscope, 37°C incubator with humidified atmosphere of 5% CO_2 (From Healforce), cytometry (From Biotech) [20].

Assay controls

(i) Medium control (medium without cells).

(ii) Negative control (medium with cells but without the experimental drug/compound).

(iii) Positive control (medium with cells treated with a known drug, Metformin; 5 mM).

Collection of plant material

Dragon's blood tree (*D. cinnabari*) resin was collected from Socotra Island (Yemen) On May 2014.

Preparation of extracts

The powdered resin was successively extracted by using 500g from resin material and dissolved with different solvents upon the polarity from non-polar to polar of the solvents (Hexane, Benzene, Diethyl ether, Dichloromethane, Chloroform, Ethyl acetate, Acetone, Ethanol, Methanol, Water) by using Soxhlet apparatuses. The exaction process started from non-polar to polar are summarized in Table 1. The extracts samples were kept at 0°C for further assays [7-19] (Table 1).

MTT assay

The amount of viable cells was determined by examining cell number with the 3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 5 mg/ml) dye-reduction assay measuring mitochondrial respiratory function. The antiproliferation (cytotoxicity) of *D. cinnabari* extracts on MCF-7 was evaluated by the MTT assay. The monolayer cell culture was trypsinized and the cell count was adjusted, using DMEM containing 10% FBS, such that 200 µl of suspension contains approximately 25,000 cells. To each well of the 96 well microtitre plate, 200 µl of the diluted cell suspension (approximately 25,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was aspirated and 200 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plate. The plate was then incubated at 37 °C for 24 h in 5% CO_2 atmosphere. After 24 h, the plate was removed from the incubator and MTT reagent was added to a final concentration of 10% of total volume. The plate wrapped with aluminum foil to avoid exposure to light and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The culture medium was aspirated without disturbing the monolayer. Then 100 µl of solubilisation solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line. The MTT assay can be used reliably to measure metabolic activity of cell cultures *in vitro* for the assessment of growth characteristics IC_{50}-values and cell survival [20].

Solvent	Resin
Hexane	12 gm
Benzene	33.5 gm
Diethyl ether	60 gm
Dichloromethane	14.8 gm
Chloroform	45 gm
Ethyl acetate	120.5 gm
Acetone	64 gm
Ethanol	55 gm
Methanol	88.2 gm
Water	5 gm
Residue	200 gm

Table 1: Shows the yield of phytochemicals in the solvent extracts of *D. cinnabari* resin.

Cell lines and culturing: MCF-7 cells were cultured in DMEM HG (Himedia) in T-75 tissue culture flask (Corning) until they reached 70% confluence. The cells were then harvested and used for the evaluation of anti-cancer activity, these steps of cell lines culturing can be represented as following:

Day 1: Cell seeding

1. Calculate the number of cells and media required and make a cell suspension:

1 well → 25,000 cells in 200 µl media

'52' wells → (25,000 × n) cells in (200 × n) µl media

Eg. 52 wells → (25000 × 52) = 1.3 × 10^6 cells in (200 × 52) = 10400 µl (or 10.4 ml) media

2. Seed the cells by pipetting 200 µl of cell suspension into each well (25,000 cells).

3. Incubate at 37°C for 24 hours.

Day 2: Drug addition

1. Make stock solution for the drug depending on the test concentrations required (Eg. 1 mg/ml)

2. Make test concentrations by serially diluting the stock (5, 25, 50, 75, 100.... µg/ml)

Dilution factor (DF) = [Stock conc.]/[Test conc]

Vol. required from stock= Total drug volume needed / DF

Eg. For stock of 1 mg/ml

If test conc required is 50 µg/ml in a total of 500 µl media

DF= 1 x 1000 µg/ml / 50 µg/ml = 20

Vol. required from stock= 500 µl / 20 = 25 µl

So, Take 25 µl of drug from stock + 475 µl of media

3. Remove media from all the wells and add the respective drug concentrations to each well.

4. Incubate at 37°C for 24 hours.

Day 3: MTT addition and reading

1. Make required volume of media containing 10% MTT.

Eg. For 54 wells

Total media required = 54 x 200 µl = 10800 µl (or 10.8 ml)

10% of 10.8 = 1080 µl

So, 1080 µl MTT + 9720 µl media

2. Remove the media from all the wells and add 200 µl MTT containing media to all the wells.

3. Incubate for 3 hours at 37°C in dark.

4. After the formazon crystals have formed remove media from all the wells and add 100 µl of DMSO to all the wells and shake the plate to dissolve the formazon crystals.

5. Measure the absorbance in a microplate reader at 570 nm [18-23].

LDH assay

LDH assays can be achieved by evaluating LDH released into the media as a marker of dead cells or performing lysis LDH as an indication of remaining live cells. Apoptosis and necrosis are two most important forms of cell death observed in normal and disease pathologies. A key signature for necrotic cells is the permeabilization of plasma membrane. This plasma membrane leakage from necrotic cells causes the release of intracellular contents into extracellular environment. In this experiment we detect the release of the enzyme Lactate dehydrogenase (LDH). LDH is a soluble cytoplasmic enzyme that is present in almost all cells and is released into extracellular space when the plasma membrane is damaged. The cells are fixed and stained with fluorescence tagged antibodies specific to this particular enzyme which is then measured by flow cytometry. Therefore, necrotic cells show decreased fluorescence intensity when compared to non-necrotic/healthy cells.

Materials

70% Ethanol (-20°C), Trypsin-EDTA solution, 1x PBS, 0.5% bovine serum albumin (BSA) in 1X phosphate-buffered saline (PBS), Mouse Anti-Lactate Dehydrogenase antibody (Abcam Catalog no. ab55433), Goat Anti-Mouse Ig FITC (BD Biosciences Catalog No. 349031).

Equipment

1. Centrifuge.

2. Pipettes. You will need one in the range of 2-10 µl, one in the range of 10-100 µl, and another ranging from 100-1000 µl.

3. Vortex mixer. You could mix by tapping or shaking the tubes, but a mixer will give much more reproducible results in most cases.

4. 12 × 75 mm polystyrene tubes.

5. Ice bucket with cover. Generally, cells are more stable and tolerate insult better when they're cold. The cover keeps light out, which could bleach the fluorochromes.

6. Flow cytometer.

Procedure

1. Culture cells in a 6-well plate at a density of 1×10^5 cells/2 ml and incubate in a CO_2 incubator overnight at 37°C for 24 hours.

2. Aspirate the spent medium and treat the cells with required concentration of experimental compounds and control in 2 ml of culture medium and incubate the cells for 24 hours.

3. At the end of the treatment, remove the medium from all the wells and give a PBS wash. Remove the PBS and add 200 µl of trypsin-EDTA solution and incubate at 37°C for 3-4, minutes. Add 2 ml culture medium and harvest the cells directly into 12 x 75 mm polystyrene tubes.

4. Centrifuge the tubes for five minutes at 300 × g at 25°C. Carefully decant the supernatant.

5. Wash twice with 1 ml PBS. Decant the PBS completely and blot lip dry.

6. Add 1 ml chilled 70% ethanol to the pellet while continuously vortexing and keep the tubes at 4°C for 15 minutes. Centrifuge the tubes for five minutes at 300 x g at 4°C. Carefully decant the supernatant.

7. Wash with 1 ml PBS. Add 1 ml of 0.5% BSA in 1X phosphate-buffered saline (PBS) and incubate for 15 minutes. Centrifuge the tubes for five minutes at 300 x g at 4°C. Carefully decant the supernatant.

8. Add 100 µl of 0.5% BSA in 1X phosphate-buffered saline (PBS) containing the Primary Antibody (Mouse Anti-Lactate Dehydrogenase antibody) at 1:100 dilution. Mix thoroughly and incubate for 30 minutes in the dark at room temperature (20°C to 25°C).

9. Wash with 1 ml of 0.5% BSA in 1X phosphate-buffered saline (PBS).

10. Add 100 µl of 0.5% BSA in 1X phosphate-buffered saline (PBS) containing the Secondary Antibody (Goat Anti-Mouse Ig FITC) at 4:100 dilution. Mix thoroughly and incubate for 30 minutes in the dark at room temperature (20°C to 25°C). 11. Wash with 1 ml PBS. Add 0.5 ml of PBS, mix thoroughly, and analyze [22-25]

Results

The results obtained from the MTT assay are as follows.

Table 2, Figures 1 and 2.

Table 3, Figures 3 and 4.

Table 4, Figures 5 and 6.

Table 5, Figures 7 and 8.

Concentration Unit: µg/ml								
	Blank	**Untreated**	**Metformin (5 mM)**	**5**	**25**	**50**	**75**	**100**
Reading 1	0.007	0.747	0.331	0.744	0.433	0.35	0.194	0.211
Reading 2	0.016	0.802	0.369	0.767	0.443	0.259	0.253	0.15
Mean	0.0115	0.7745	0.35	0.7555	0.438	0.3045	0.2235	0.1805
Mean OD-Mean B	NA	0.763	0.3385	0.744	0.4265	0.293	0.212	0.169
SD		0.016263456	0.007071068	0.0070711	0.007071	0.064347	0.041719	0.043134
Standard error		0.011501737	0.005000755	0.0050008	0.005001	0.045507	0.029504	0.030505
Viability %	NA	100	44.36435125	97.50983	55.89777	38.40105	27.78506	22.14941
IC50 = 48.117 µg/ml								

Table 2: The percentage viability of the cells obtained from the cytotoxicity studies at IC50 = 48.117 µg/ml.

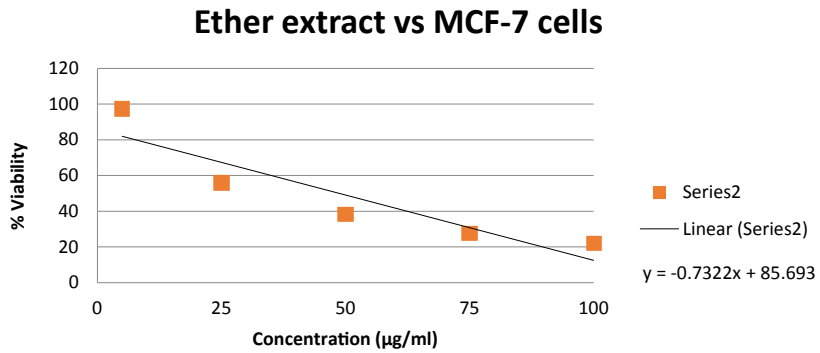


Figure 1: Ether extract vs. MCF-7 cells.

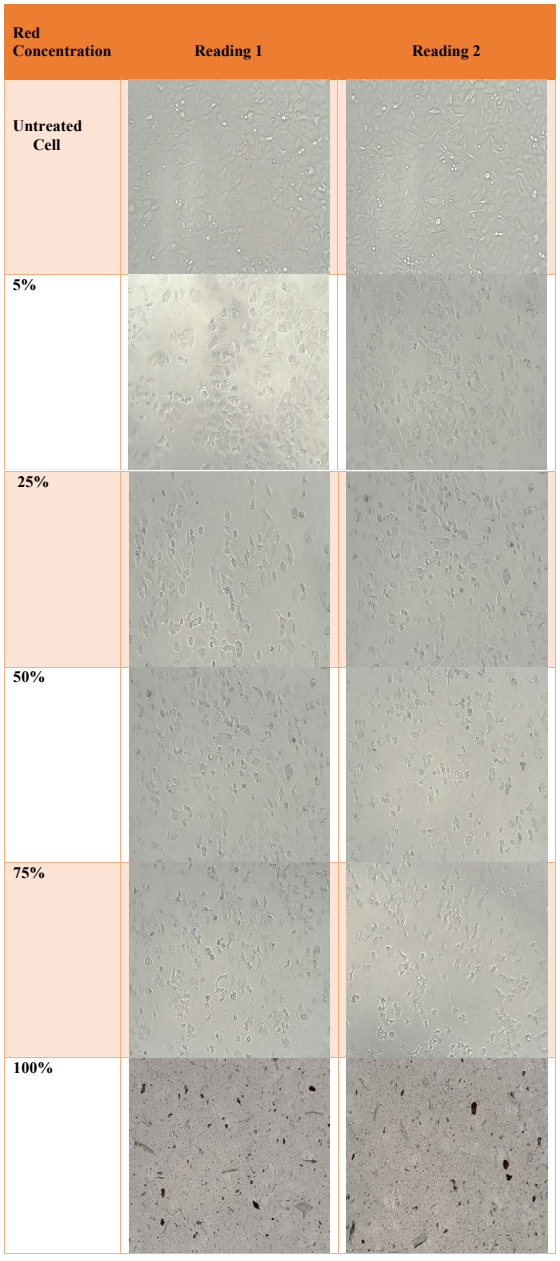


Figure 2: The cytotoxicity studies revealed that the half maximal inhibitory concentration (IC50) for the ether extract is 48.117 µg/ml.

Concentration Unit: µg/ml								
	Blank	**Untreated**	**Metformin (5 mM)**	**5**	**25**	**50**	**75**	**100**
Reading 1	0.007	0.747	0.331	0.587	0.508	0.423	0.249	0.206
Reading 2	0.016	0.802	0.369	0.541	0.515	0.378	0.285	0.249
Mean	0.0115	0.7745	0.35	0.564	0.5115	0.4005	0.267	0.2275
Mean OD-Mean B	NA	0.763	0.3385	0.5525	0.5	0.389	0.2555	0.216
SD		0.032526912	0.004949747	0.0049497	0.00495	0.03182	0.025456	0.030406
Standard Error		0.023003474	0.003500529	0.0035005	0.003501	0.022503	0.018003	0.021503
Viability %	NA	100	44.36435125	72.411533	65.5308	50.98296	33.48624	28.30931
IC50 = 50.692 µg/ml								

Table 3: The percentage viability of the cells obtained from the cytotoxicity studies at IC50 = 50.692 µg/ml.

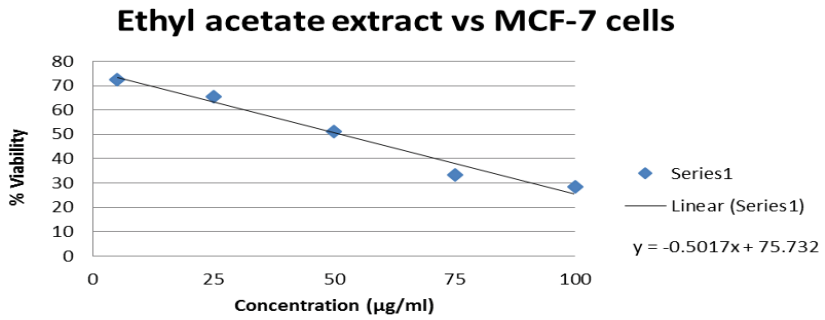


Figure 3: Ethyl acetate vs. MCF-7 cells.

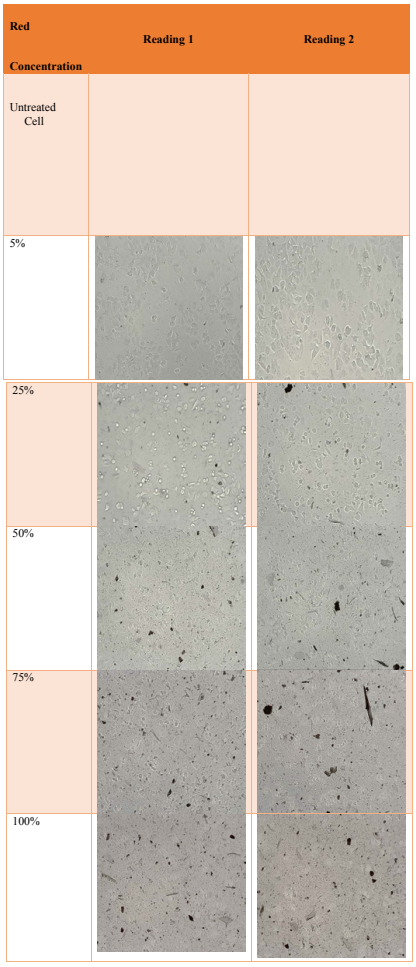


Figure 4: The cytotoxicity studies revealed that the most half maximal inhibitory concentration (IC50) for the Ethyl acetate extract is 50.692 µg/ml.

Concentration Unit: µg/ml								
	Blank	**Untreated**	**Metformin (5 mM)**	**5**	**25**	**50**	**75**	**100**
Reading 1	0.007	0.747	0.331	0.761	0.614	0.497	0.498	0.367
Reading 2	0.016	0.802	0.369	0.733	0.65	0.525	0.433	0.4
Mean	0.0115	0.7745	0.35	0.747	0.632	0.511	0.4655	0.3835
Mean OD-Mean B	NA	0.763	0.3385	0.7355	0.6205	0.4995	0.454	0.372
SD		0.01979899	0.025455844	0.0254558	0.025456	0.019799	0.045962	0.023335
Standard error		0.014002114	0.018002719	0.0180027	0.018003	0.014002	0.032505	0.016502
Viability %	NA	100	44.36435125	96.395806	81.32372	65.46527	59.50197	48.75491
IC50 = 93.1136 µg/ml								

Table 4: The percentage viability of the cells obtained from the cytotoxicity studies at IC50 = 93.1136 µg/ml.

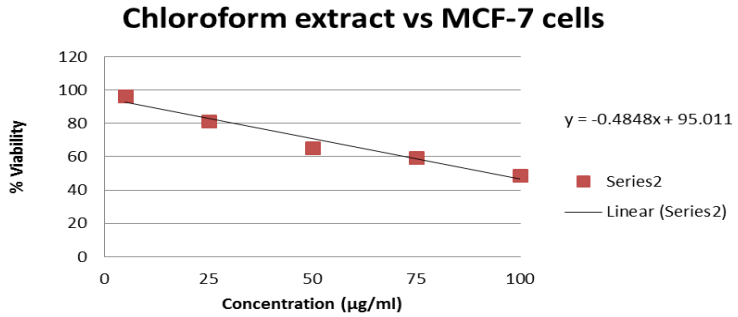


Figure 5: Chloroform vs. MCF-7 cells.

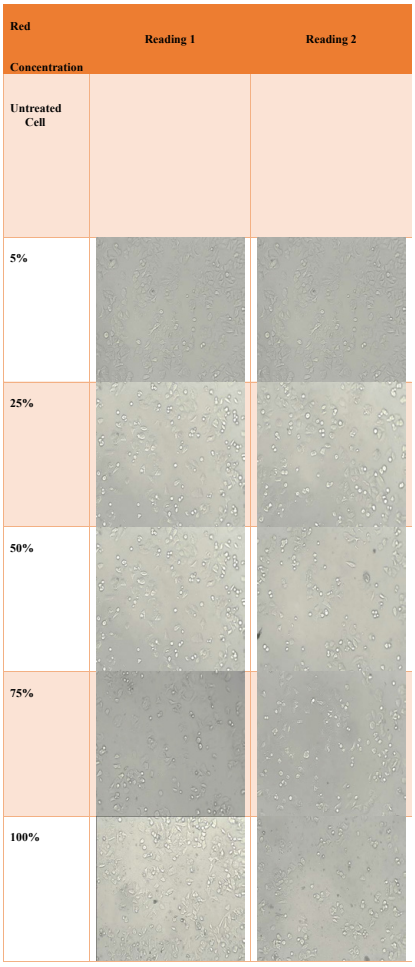


Figure 6: The cytotoxicity studies revealed that the minimum inhibitory concentration (IC50) for the Chloroform extract is 93.1136 µg/ml.

Concentration Unit: µg/ml								
	Blank	**Untreated**	**Metformin (5mM)**	**5**	**25**	**50**	**75**	**100**
Reading 1	0.007	0.747	0.331	0.654	0.675	0.602	0.561	0.414
Reading 2	0.016	0.802	0.369	0.752	0.726	0.619	0.512	0.404
Mean	0.0115	0.7745	0.35	0.703	0.7005	0.6105	0.5365	0.409
Mean OD-Mean B	NA	0.763	0.3385	0.6915	0.689	0.599	0.525	0.3975
SD		0.069296465	0.036062446	0.0360624	0.036062	0.012021	0.034648	0.007071
Standard error		0.049007401	0.025503851	0.0255039	0.025504	0.008501	0.024504	0.005001
Viability %	NA	100	44.36435125	90.629096	90.30144	78.5059	68.80734	52.09699
IC50 = 115.218 µg/ml								

Table 5: The percentage viability of the cells obtained from the cytotoxicity studies at IC50 = 115.218 µg/ml.

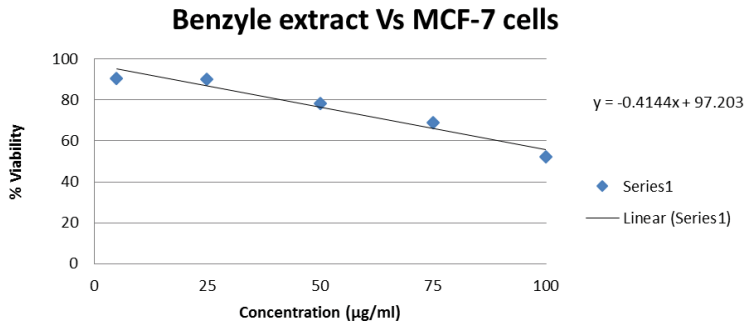


Figure 7: Benzene vs. MCF-7 cells.

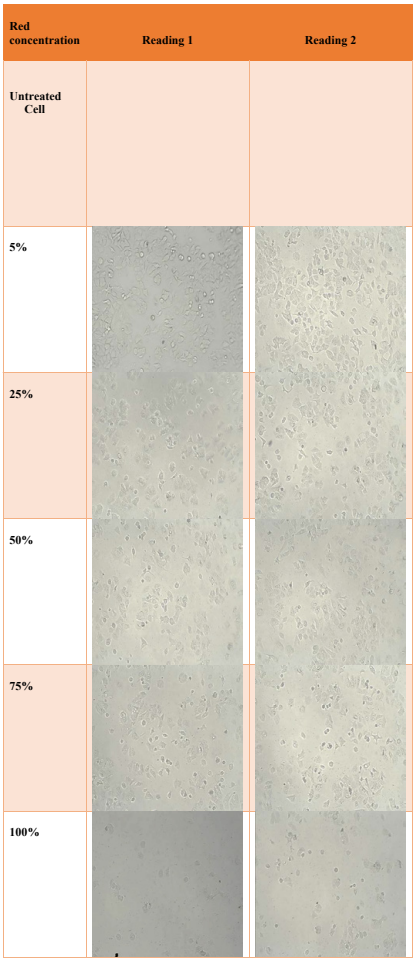


Figure 8: The cytotoxicity studies revealed that the half minimum inhibitory concentration (IC50) for the benzene extract is 115.218 µg/ml which is less than chloroform extract.

Concentration Unit: µg/ml								
	Blank	**Untreated**	**Metformin (5 mM)**	**5**	**25**	**50**	**75**	**100**
Reading 1	0.007	0.747	0.331	0.706	0.703	0.532	0.504	0.582
Reading 2	0.016	0.802	0.369	0.742	0.71	0.632	0.584	0.635
Mean	0.0115	0.7745	0.35	0.724	0.7065	0.582	0.544	0.6085
Mean OD-Mean B	NA	0.763	0.3385	0.7125	0.695	0.5705	0.5325	0.597
SD		0.025455844	0.004949747	0.0049497	0.00495	0.070711	0.056569	0.037477
Standard error		0.018002719	0.003500529	0.0035005	0.003501	0.050008	0.040006	0.026504
Viability %	NA	100	44.36435125	93.381389	91.08781	74.77064	69.7903	78.24377
IC50 = 200.8177 µg/ml								

Table 6: The percentage viability of the cells obtained from the cytotoxicity studies at IC50= 200.8177 µg/ml.

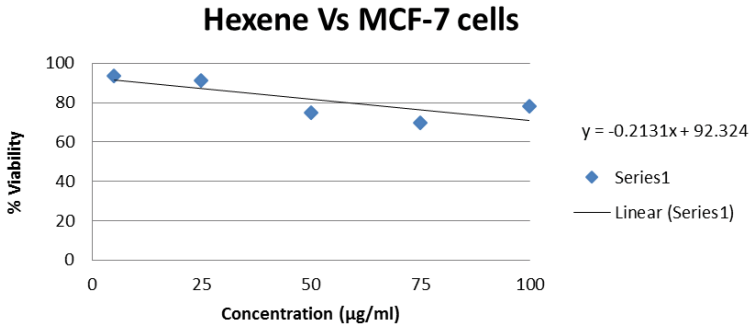


Figure 9: Hexane vs. MCF-7 cells.

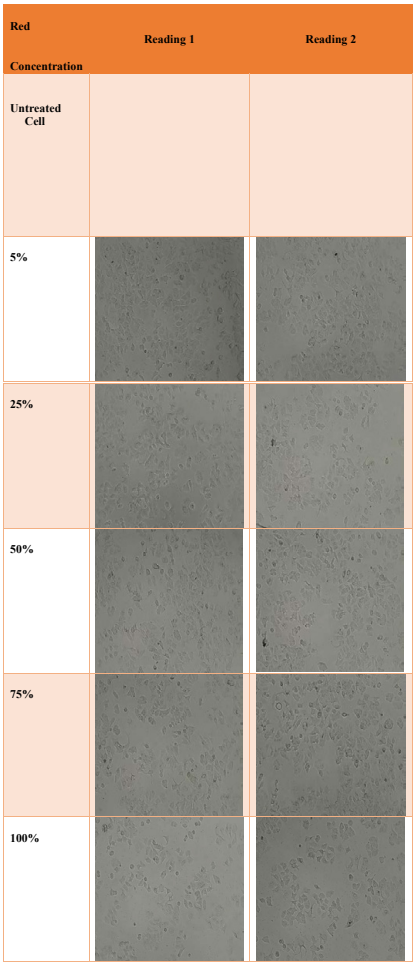
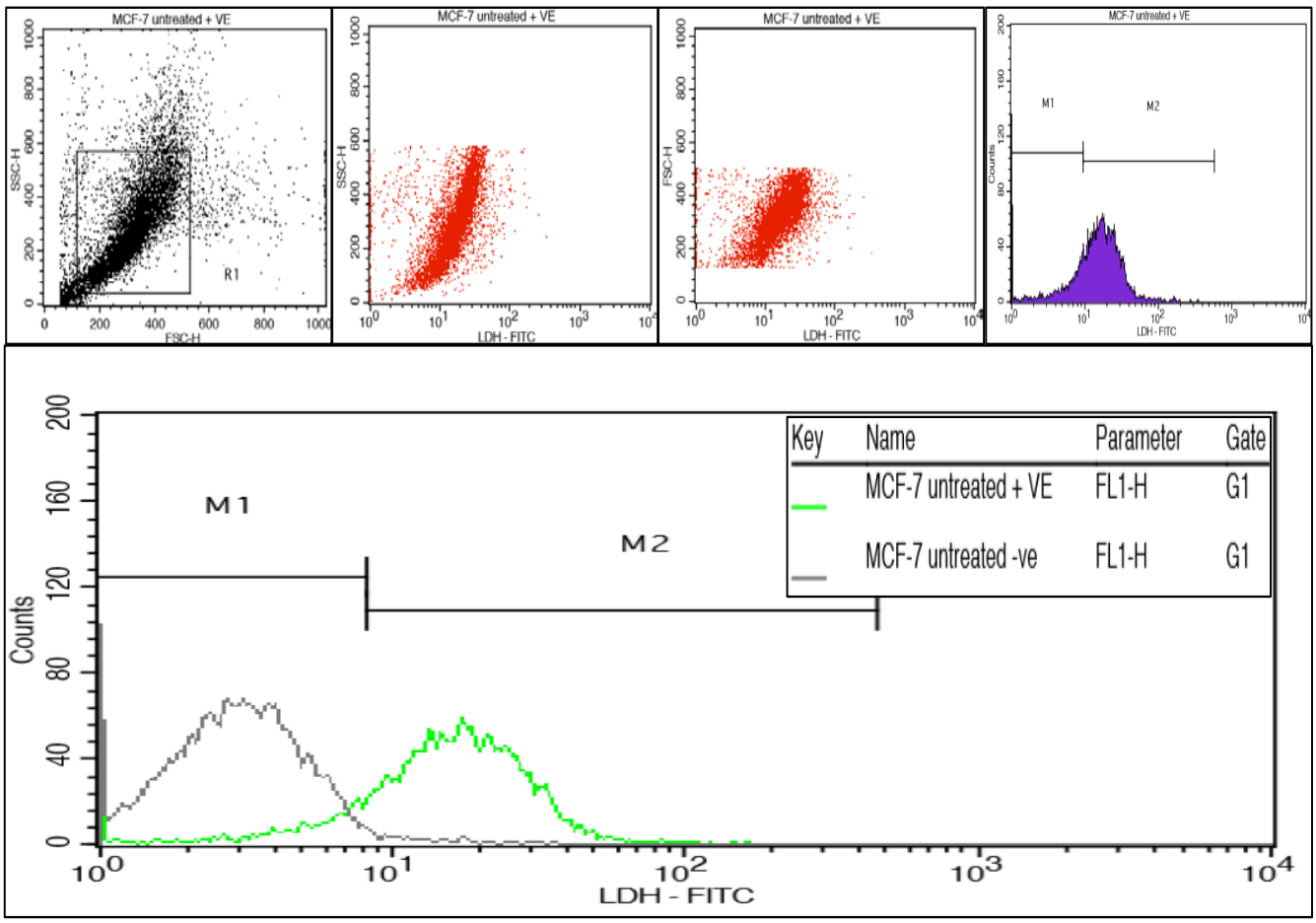


Figure 10: The cytotoxicity studies revealed that the minimum inhibitory concentration (IC50) for the hexane extract is 93.1136 µg/ml which is the least extract.

Untreated Positive Control



Histogram Statistics

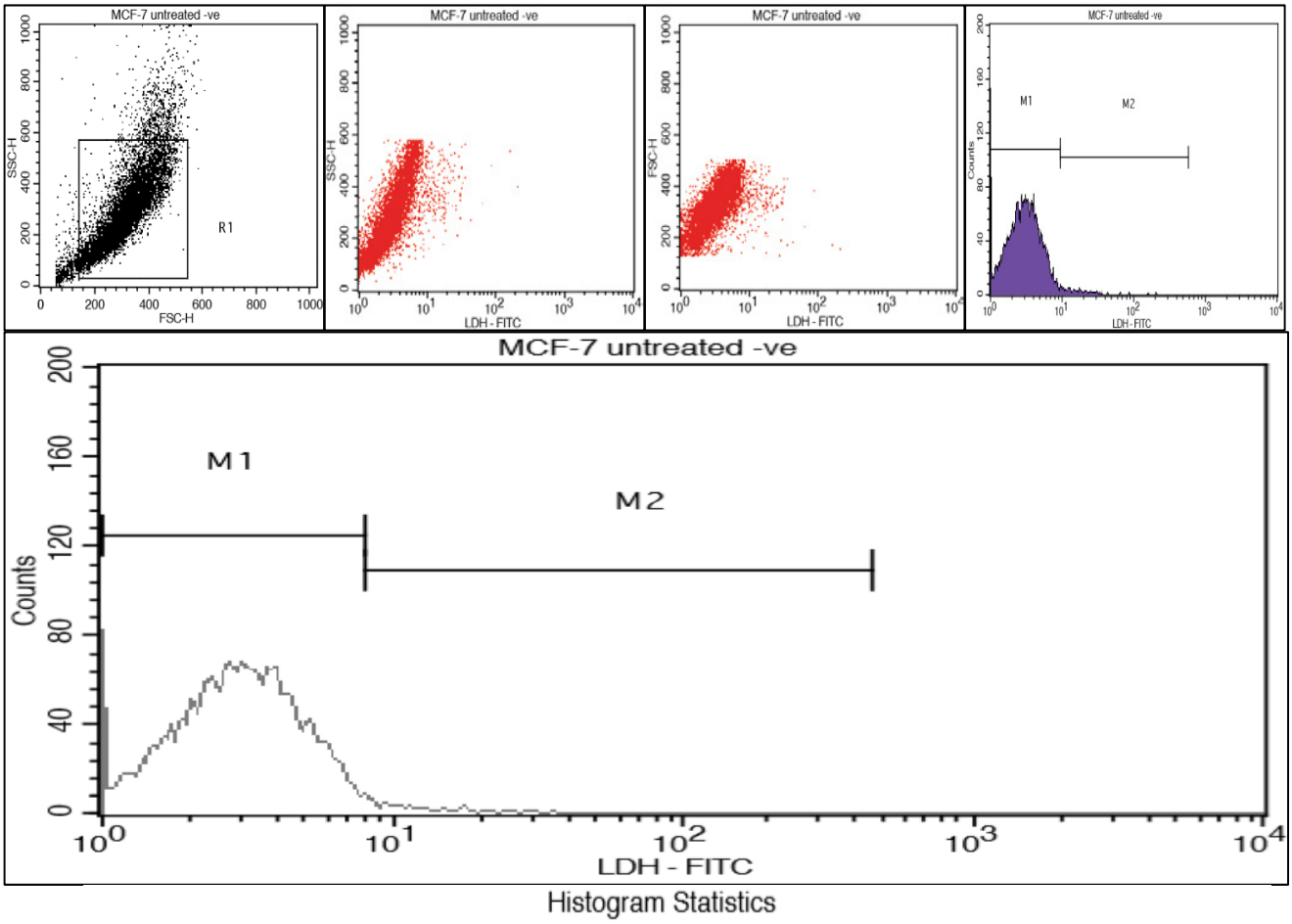
File: MCF-7 untreated + VE
Sample ID: MCF-7 untreated + VE
Tube: Untitled
Acquisition Date: 24-Jun-15
Gated Events: 7573
X Parameter: LDH - FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: G1
Total Events: 9420

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	7559	100.00	80.24	17.87	14.39	72.59	15.82	1
M1	1, 8	1152	15.24	12.23	4.93	4.11	47.47	5.38	1
M2	8, 445	6448	85.30	68.45	20.11	17.94	63.25	17.47	17

Figure 11: Untreated LDH FITC stained cells appeared in the M2 region (85.3%) as LDH positive cells with increased FITC fluorescence intensity.

Unstained Negative Control



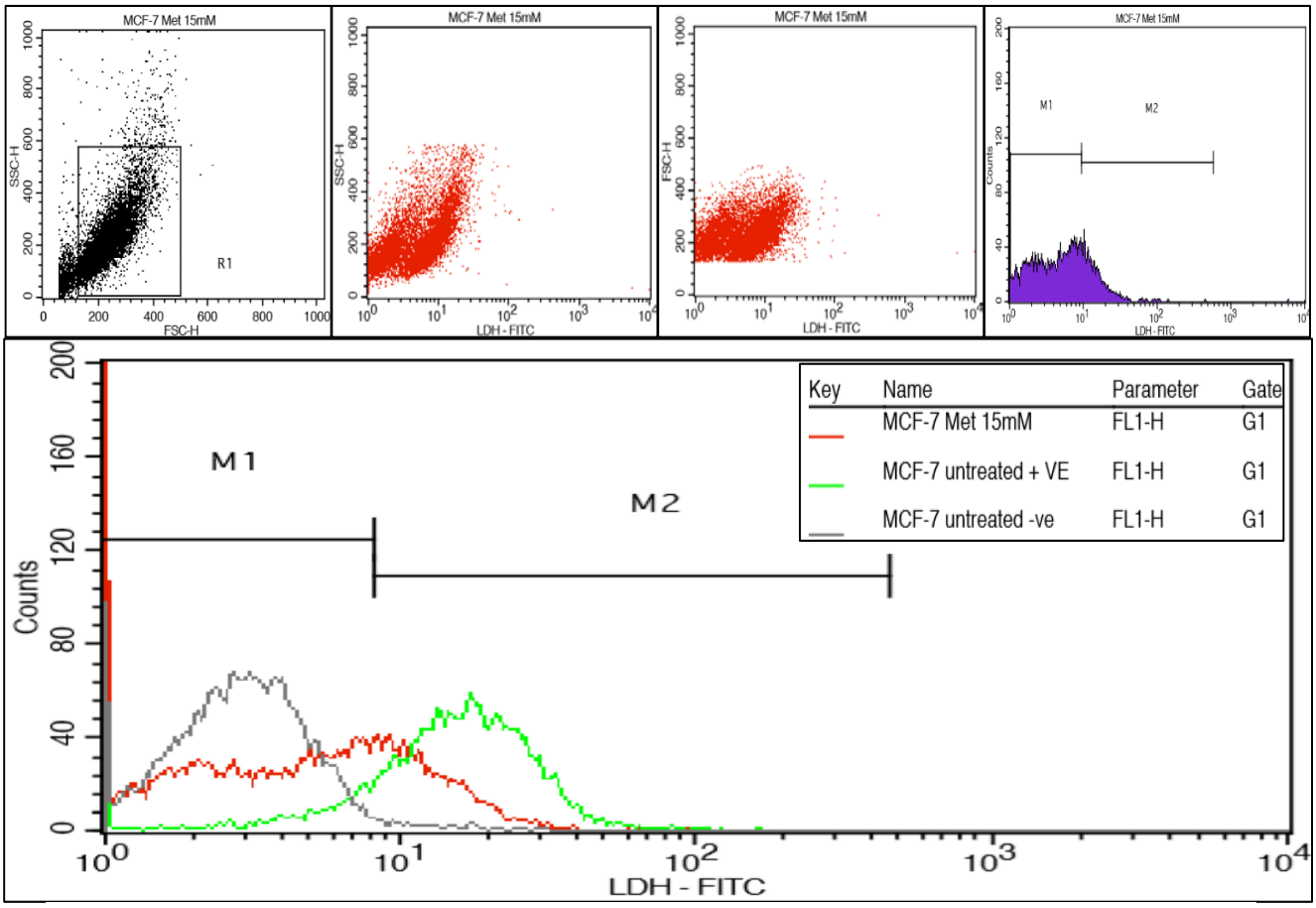
Histogram Statistics

File: MCF-7 untreated -ve	Log Data Units: Linear Values
Sample ID: MCF-7 untreated -ve	Patient ID:
Tube: Untitled	Panel: Untitled Acquisition Tube List
Acquisition Date: 24-Jun-15	Gate: G1
Gated Events: 8872	Total Events: 10000
X Parameter: LDH - FITC (Log)	

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	8868	100.00	88.68	3.58	3.04	107.25	3.05	1
M1	1, 8	8639	97.42	86.39	3.25	2.93	45.47	3.00	1
M2	8, 445	241	2.72	2.41	15.83	13.15	111.93	11.24	8

Figure 12: The Unstained LDH FITC negative cells were put in the M1 region (97.42%).

Metformin



Histogram Statistics

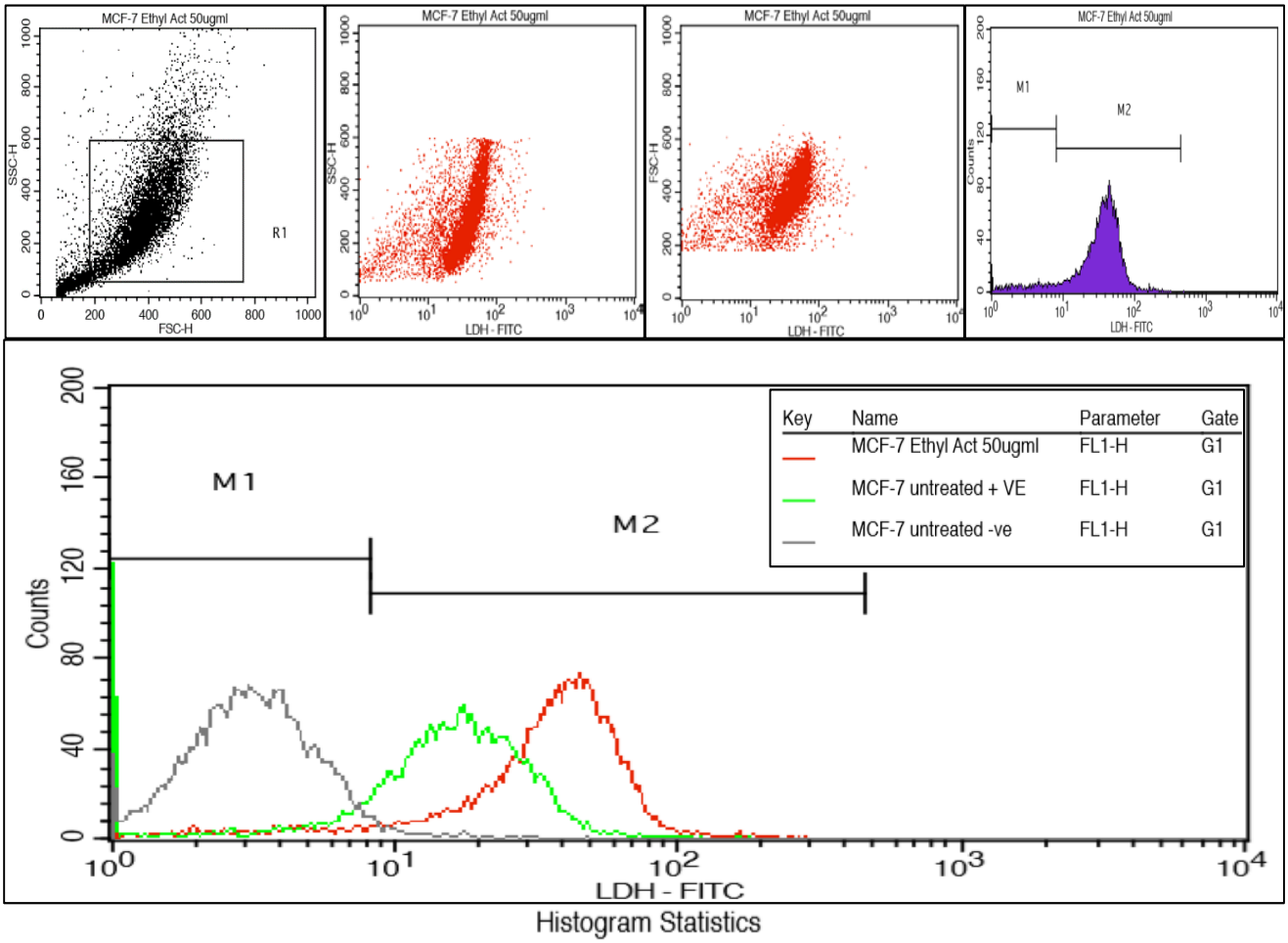
File: MCF-7 Met 15mM
Sample ID: MCF-7 Met 15mM
Tube: Untitled
Acquisition Date: 24-Jun-15
Gated Events: 8689
X Parameter: LDH - FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	8693	100.00	86.93	8.46	4.64	1437.30	5.05	1
M1	1, 8	6083	69.98	60.83	3.65	3.01	58.55	3.08	1
M2	8, 445	2678	30.81	26.78	13.59	12.48	79.36	11.55	10

Figure 13: Metformin treated cells showed a decrease in FITC fluorescence intensity and hence a decrease in LDH with more cells in the M1 region (69.98%) than the M2 region (30.82%).

Ethyl acetate extract



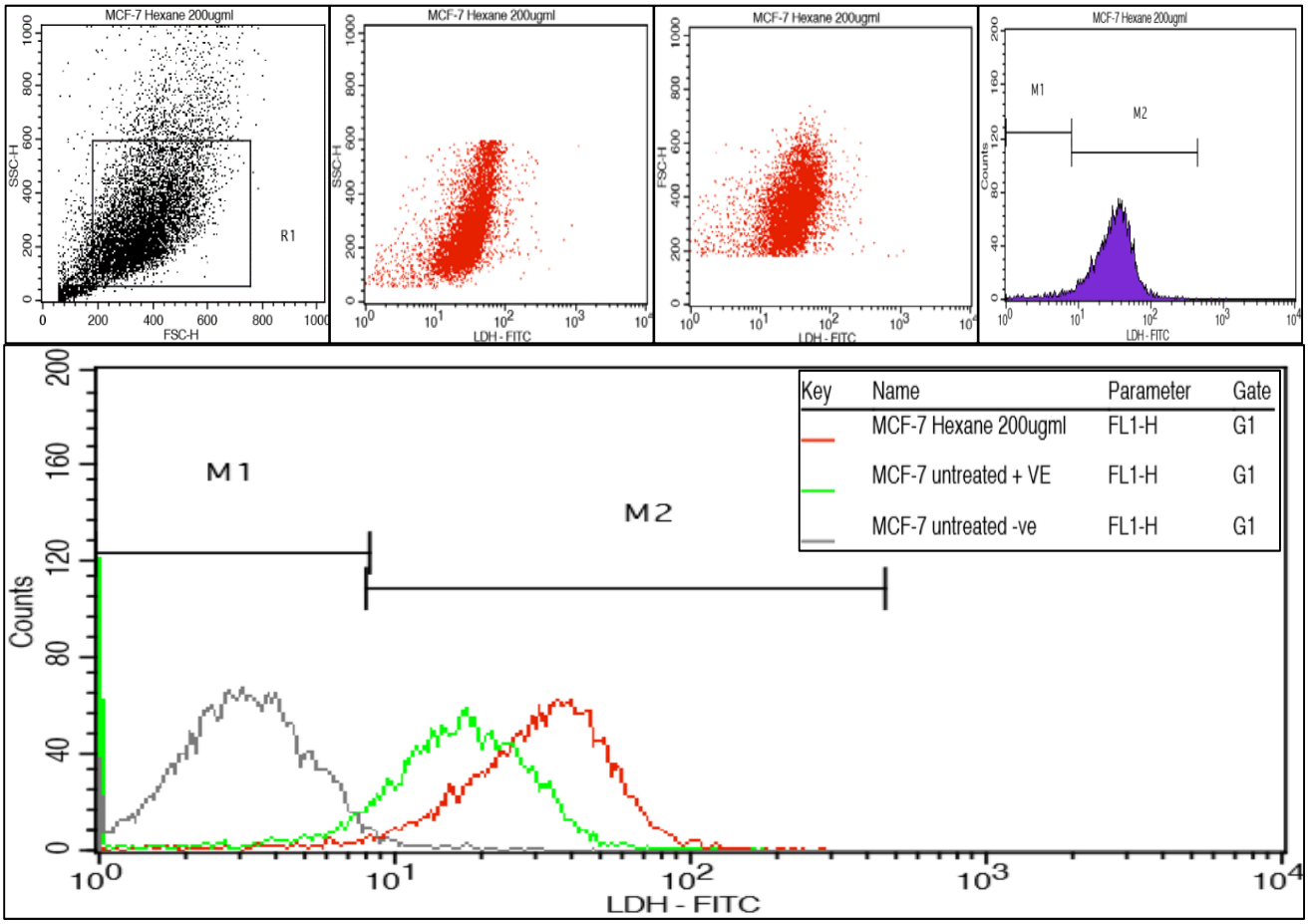
Histogram Statistics

File: MCF-7 Ethyl Act 50ugml
Log Data Units: Linear Values
Sample ID: MCF-7 Ethyl Act 50ugml
Patient ID:
Tube: Untitled
Panel: Untitled Acquisition Tube List
Acquisition Date: 30-Jun-15
Gate: G1
Gated Events: 8015
Total Events: 10000
X Parameter: LDH - FITC (Log)

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	7996	100.00	79.96	37.97	30.46	60.99	36.85	44
M1	1, 8	571	7.14	5.71	3.85	3.28	53.05	3.65	1
M2	8, 445	7430	92.92	74.30	40.51	36.10	52.66	38.20	44

Figure 14: Ethyl acetate extract did not show any significant decrease in LDH.

Hexane extract



Histogram Statistics

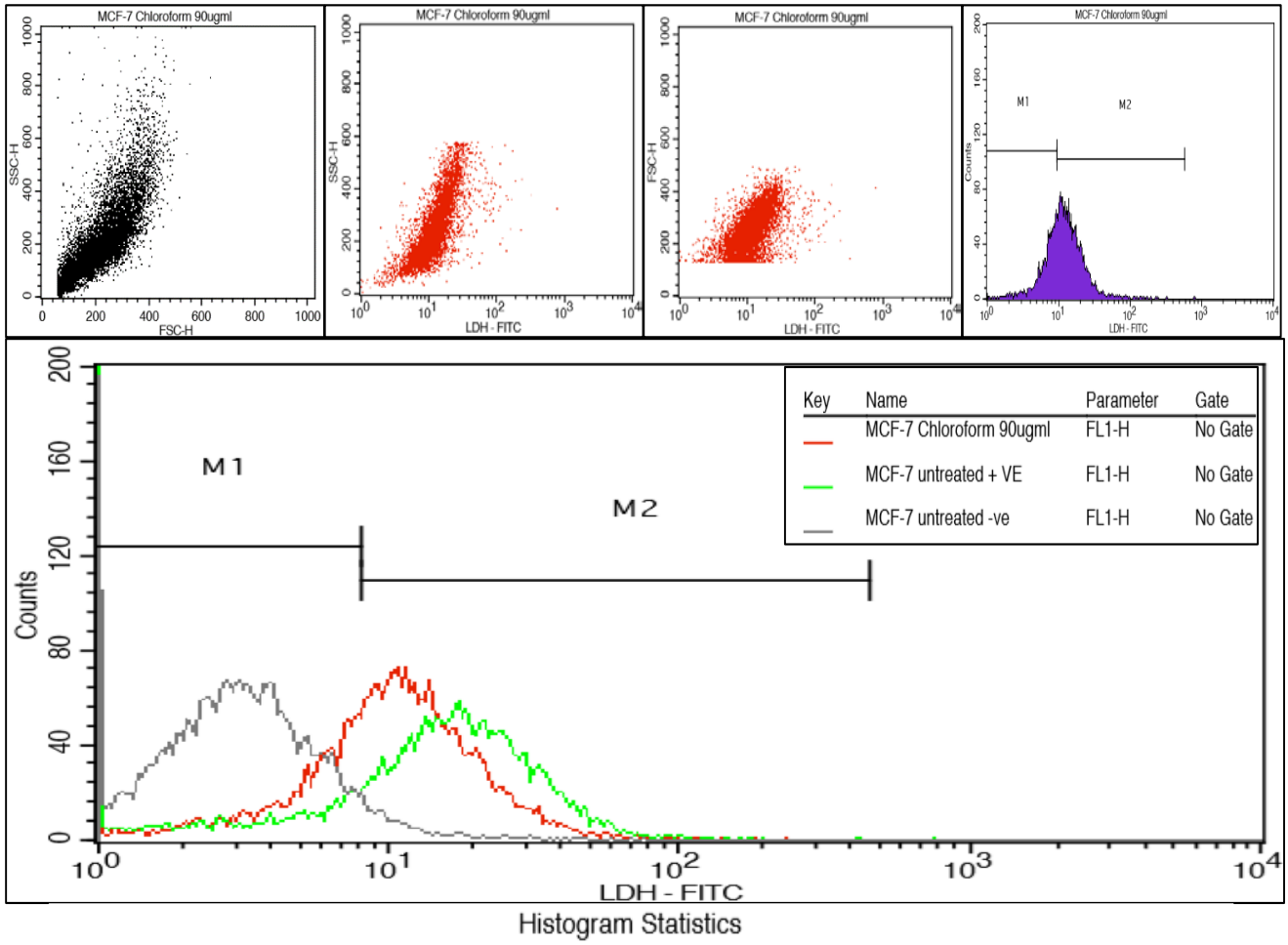
File: MCF-7 Hexane 200ugml
Sample ID: MCF-7 Hexane 200ugml
Tube: Untitled
Acquisition Date: 30-Jun-15
Gated Events: 7961
X Parameter: LDH - FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: G1
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	7951	100.00	79.51	34.73	28.83	78.91	31.62	35
M1	1, 8	309	3.89	3.09	4.59	3.98	47.79	4.49	5
M2	8, 445	7651	96.23	76.51	35.58	31.13	60.17	32.49	35

Figure 15: Hexane extract also did not show any significant decrease in LDH.

Chloroform extract



Histogram Statistics

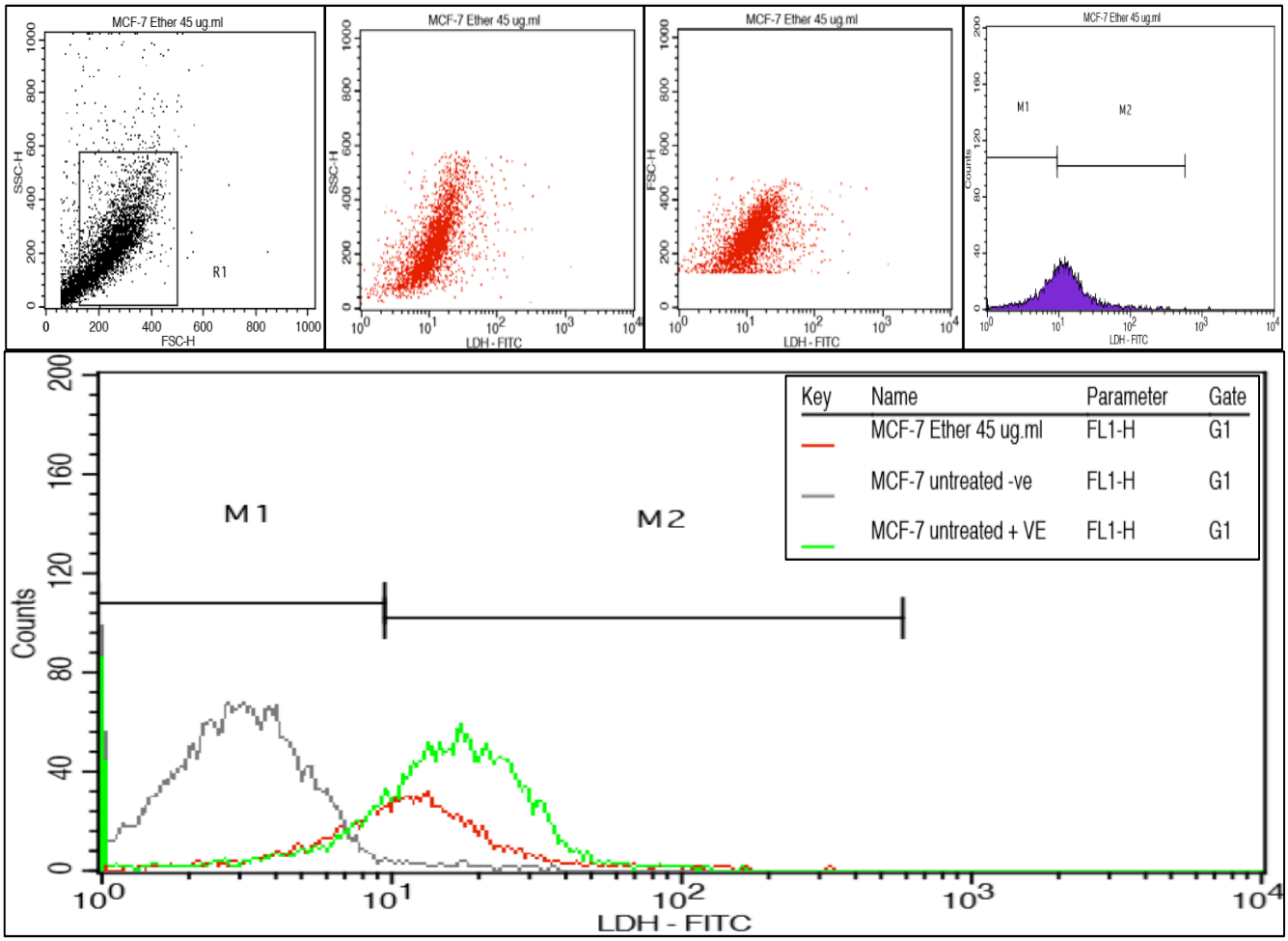
File: MCF-7 Chloroform 90ugml
Sample ID: MCF-7 Chloroform 90ugml
Tube: Untitled
Acquisition Date: 24-Jun-15
Gated Events: 10013
X Parameter: LDH - FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	14.88	10.01	809.33	10.37	10
M1	1, 8	3304	33.04	33.04	5.23	4.68	39.10	5.62	1
M2	8, 445	6774	67.74	67.74	16.55	14.42	88.26	13.10	10

Figure 16: Chloroform extract treated cells showed a decrease in LDH with 33.04% cells in the M1 region and 67.74% cells in the M2 region.

Ether extract



Histogram Statistics

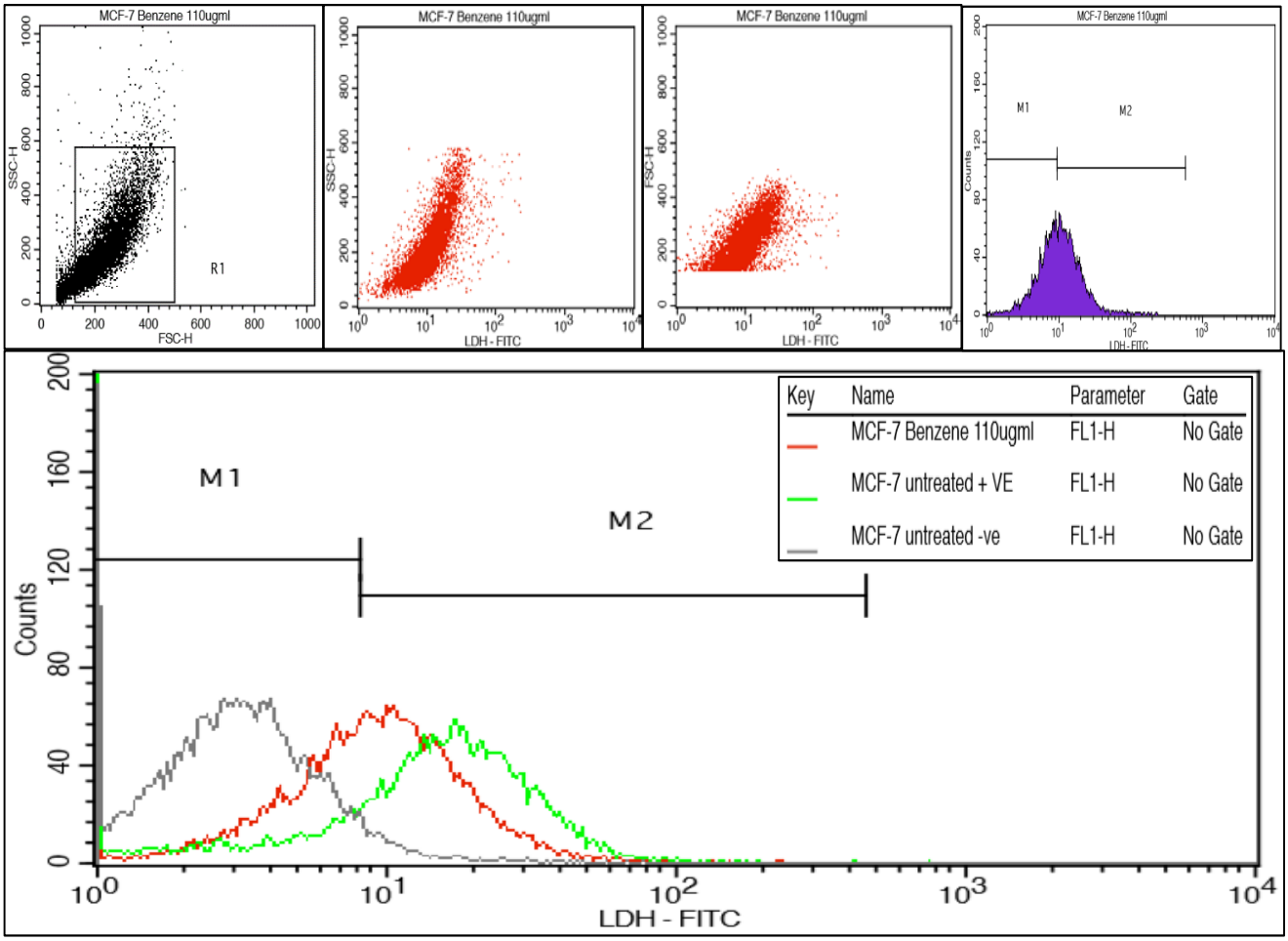
File: MCF-7 Ether 45 ug.ml
Sample ID: MCF-7 Ether 45 ug.ml
Tube: Untitled
Acquisition Date: 24-Jun-15
Gated Events: 3868
X Parameter: LDH - FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: G1
Total Events: 5040

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	3862	100.00	76.63	14.90	11.17	182.68	11.24	11
M1	1, 9	1430	37.03	28.37	6.36	5.89	33.38	6.67	8
M2	9, 578	2460	63.70	48.81	19.32	16.13	119.28	14.39	11

Figure 17: Ether extract also showed a decrease in LDH with 37.03% cells in the M1 region and 63.70% cells in the M2 region.

Benzene extract



Histogram Statistics

File: MCF-7 Benzene 110ugml
Sample ID: MCF-7 Benzene 110ugml
Tube: Untitled
Acquisition Date: 24-Jun-15
Gated Events: 9998
X Parameter: LDH - FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: No Gate
Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	14.88	9.06	1094.26	9.31	8
M1	1, 8	4158	41.58	41.58	5.22	4.78	36.14	5.47	6
M2	8, 445	5949	59.49	59.49	16.12	14.08	87.69	12.86	8

Figure 18: Benezene extract treated cells showed a decrease in LDH with 41.58% cells in the M1 region and 59.49% cells in the M2 region.

Table 6, Figures 9 and 10.

Figures 11-18.

Discussion and Conclusion

Sample collected in this study was selected to include the plant resin that have suggested bioactivity on the basis of their non-reported traditional usage as medicines. The plant resin used on traditional treatments for various disease as fever, tonsillitis, cough, dysentery, diarrhea, skin disease. The major aim of this study was to identify potential anticancer extracts that were affective not by feature of high concentration alone, relatively by specific activity demonstrated even at low doses. In order to achieve this aim, the maximum concentration (µg/ml) used in the study was 100 µg/ml as above results as the criteria for identifying plant resin with potent activity within range. Plants with less than 50% inhibitory activity within the test range were excluded from father screening. The concentration that causes 50% inhibition of cancer cells by the crude extract of the dragon blood resin displayed. Screening of Ether and Ethyl acetate of dragon cinnabari Balf. F showed the cytotoxicity studies revealed that the half minimum inhibitory concentration (IC_{50}) for the Ether and Ethyl acetate extract are 48.117, 50.692 µg/ml respectively, which resulted in moderate anticancer activities against MCF-7 cell lines, while the benzene extract showed IC_{50} 115.218 µg/ml which is less than ether and ethyl acetate extract extract with IC_{50} extract against MCF-7 cell lines, as well as the hexane extract revealed that the IC_{50} is 93.1136 µg/ml which is the least extract. The inhibitory properties of these extracts are compared with standard Metformin for MCF-7 cell line. The Percentage cancer cell inhibition profiles were found to be concentration dependent. Based on the cytotoxicity studies against MCF-7 cell lines the Ether and Ethyl acetate extracts could be used as potential source for anticancer drugs. On the other hand, Benzene, Chloroform and Ether extract treated cells showed a significant decrease in LDH FITC fluorescence intensity due to LDH leakage which indicates a necrotic cell death mechanism. While, Ethyl acetate and Hexane extract treated cells showed high LDH FITC fluorescence intensity which indicates an intact cell membrane and a possible apoptotic cell death mechanism.

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