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1 TEST REQUIREMENT:

- In vitro Screening of anti-cancer adjuvant activity of Dietary Supplement
- The samples to be tested as follows:

S. No	Customer Name	Compound Name	Cell Line
1	**HOLIN AWARD NUTRACEUTICALS LLP CHENNAI**	**KARROVITA (ONCO^{+})**	**MCF 7**

2 INTRODUCTION:

Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability.

2.1AIM:

To determine the Anti-cancer adjuvant of test compound in vitro by MTT Assay.

2.2 MATERIALS AND METHODS:

DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from eppndorf India.

2.2.1 Maintenance of Cell Line:

The Cancer cell line were purchased from NCCS, Pune and the cells were maintained in MEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 ^{0}C.

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2.2.2 Preparation of Test Compound:

For MTT assay, Each Test compounds were weighed separately and dissolved in DCM. With media make up the final concentration to 1 mg/ ml and the cells were treated with series of concentrations from 10 to 100 µg/ ml.

3 MTT ASSAY:

3.1 Principle:

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and, on the assumption, that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple coloured formazan crystals. The cells are then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

3.2 Procedure:

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and preform the thetryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^{3} cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 ^{0} C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of karrovita with standard cisplatin in represented wells in 96 plates. After 48 hrs, discard the media solution and add the fresh media with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37 ^{0} C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The IC_{50} value was determined by using linear regression equation i.e. y = mx+c. Here, y = 50, m and c values were derived from the viability graph.

4 RESULT:

Test compound treated with MCF 7cells showing the IC_{50} values are as follow in the table provided and karrovita combination with standard drug cisplatin shows the decreased IC_{50} value compared with cisplatin alone.

S. No	SAMPLE NAME	IC_{50} (µg)
1	Cisplatin	4.96 ± 0.141
2	KARROVITA	96.25±2.37
3	Cisplatin + KARROVITA	2.48±0.073

- Raw data and graphs are provided in Excel sheet separated in the file name **Cosmic-Anti cacner.XLS**

5 REFERENCE

1. A. Venkanna, B. Siva, B. Poornima, P.R. Rao Vadaparthi, K. Rajendra Prasad, K. Ashok Reddy, G. Bhanu Prakash Reddy, K. Suresh Babu, Phytochemical investigation of sesquiterpenes from the fruits of Schisandrachinensis and their cytotoxic activity, Fitoterapia 95 (2014) 102–108.

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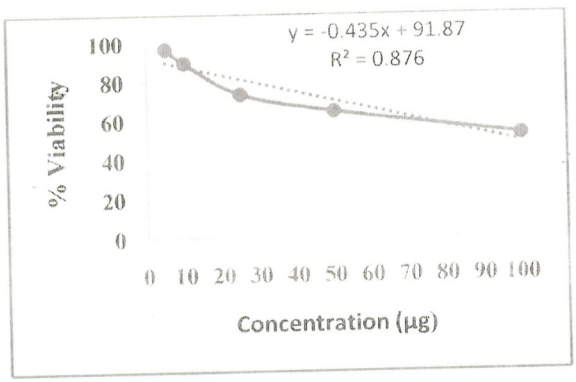
1 KARROVITA

Concentration (µg)	Absorbance at 570nm	% Inhibition	% Viability	IC_{50} (µg)
5	0.714	3.25	96.75	
10	0.661	10.43	89.57	
25	0.541	26.69	73.31	
50	0.476	35.5	64.5	
100	0.387	47.56	52.44	
Untreated	0.738	0	100	96.25±2.37
Blank	0	0	0	



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2 CISPLATIN +KARROVITA

Concentration (µg)	Absorbance at 570nm	% Inhibition	% Viability	IC_{50} (µg)
5	0.42	43.08	56.92	
10	0.331	55.14	44.86	
25	0.248	66.39	33.61	
50	0.138	81.3	18.7	
100	0.112	84.82	15.18	
Untreated	0.738	0	100	2.48±0.073
Blank	0	0	0