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1. Introduction

The present work describes the precise, accurate and reproducible cytotoxicity assay (MTT) on different types of cell lines. The MTT assay is used to assess the metabolic activity or viability of cells in microcapsules. A water-soluble dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, can be converted by metabolically active cells into an insoluble formazan. An estimation of the number of cells in capsules can be obtained through the quantitative measurement of formazan.

A specified population of cells that can be kept in culture for an extended period of time while keeping stability in their phenotypes and functions is referred to as a cell line in general. Cell lines are often clonal, which means that their whole population descended from a single cell that served as its common ancestor.

2. Methodology

2.1 Drug preparation

The sample was received from herbal home company. It was oil with unknown mixture.

The sample was water insoluble. 2ml from oil sample was mixed with 2 ml DMSO (ratio 1:1) and vortex then DMEM media was added to reach final 10 ml. After that; the sample left for 2 hrs until the oil layer separated and removed totally. The lower layer was taken as a stock with final concentration 200 ul/ml. serial dilution (1:1) was done to reach final concentration 1.78 ul/ml.

2.2 Cell culture

Ten cell lines from different types of cancer were used in this experiment. Cell lines were cultured as monolayer in aproppret media (DMEM/ RPMI) supplemented with 10% FBS and 100 U/mL penicillin- streptomycin and incubated in a humidified 5% CO_2 atmosphere at 37 ^oC.

2.3 MTT Assay

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) (Promega, USA) assay was used to measure oily extract sample cytotoxicity in cell lines. Nine cell lines were chosen (table 2.3.3). 20,000 cell/ well were seeded in triplicates into 96 well tissue culture plate and incubated overnight at 37°C with 5% CO_2. At the second day, 100 µl from serially diluted extract was added into each well (200 µl to 1.56 µl) and incubated for 24, 48 and 72 hrs at 37°C with 5% CO_2. After the incubation; according to the promega manufactural protocol; 15 µl MTT dye was added to each well and incubated for 3 hrs in incubator at 37°C. Then, 100 µl stop day was added and incubated with shaking for 45 min. Color development was measured using spectrophotometer at 570 nm.

This work was done triplicate (three trials).

2.3 Reagents, chemicals and cell lines

2.3.1 Reagents and chemicals

Description	Source
DMSO	SolMate
DMEM media	Euro clone
MTT kit	Promega
Fetal bovine serum (FBS)	Cytiva
Penicillin- streptomycin	Euro clone
Trypsin-EDTA	Euro clone

2.3.2 Instrumentations

Instrument	Source
Water bath	Selecta
Biosafety cabinet	Thermo Scientific
Centrifuge	Electra Mdeical
Micro plate reader	BioTek
Co2 Incubator	Binder
Cell counter	Accuris
Microscope	Leica

2.3.2 Cell lines

Cell line	Full Name	Source
MDA MB 231	Human breast cancer	ATCC
MCF7	Human breast cancer	ATCC
HT-29	Human colorectal adenocarcinoma	ATCC
A549	Human lung adenocarcinoma	ATCC
K562	Human Chronic Myelogenous Leukemia	ATCC
EA.HY926	Hybridoma line derived from human endothelium and A549/8 cells	ATCC
HepG2	Human liver cancer	ATCC
PANC-1	Human Pancreatic Cancer	ATCC
MIA PACA2	Human Pancreatic Cancer	ATCC

3. Result

3.1 HepG2 cell line

A 15-year-old Caucasian male with a hepatocellular carcinoma donated his liver tissue to create the human liver cancer cell line known as Hep-G2.

The cytotoxicity of oily extract sample was also investigated on HepG2 cell line. As shown in figure 1 and table 1; the half-maximal inhibitory concentration (IC_{50}) value at 24, 48 and 72 hr was 1.704, 1.332and 0.7879 respectively. The best treatment time was 24 hr so the trial was repeated twice. Moreover, the result show that; there was no significant difference between the three trails (figure 3). The IC_{50} for the second and the third trial was 5.492 µl and 4.437µl; respectively (figure 2).

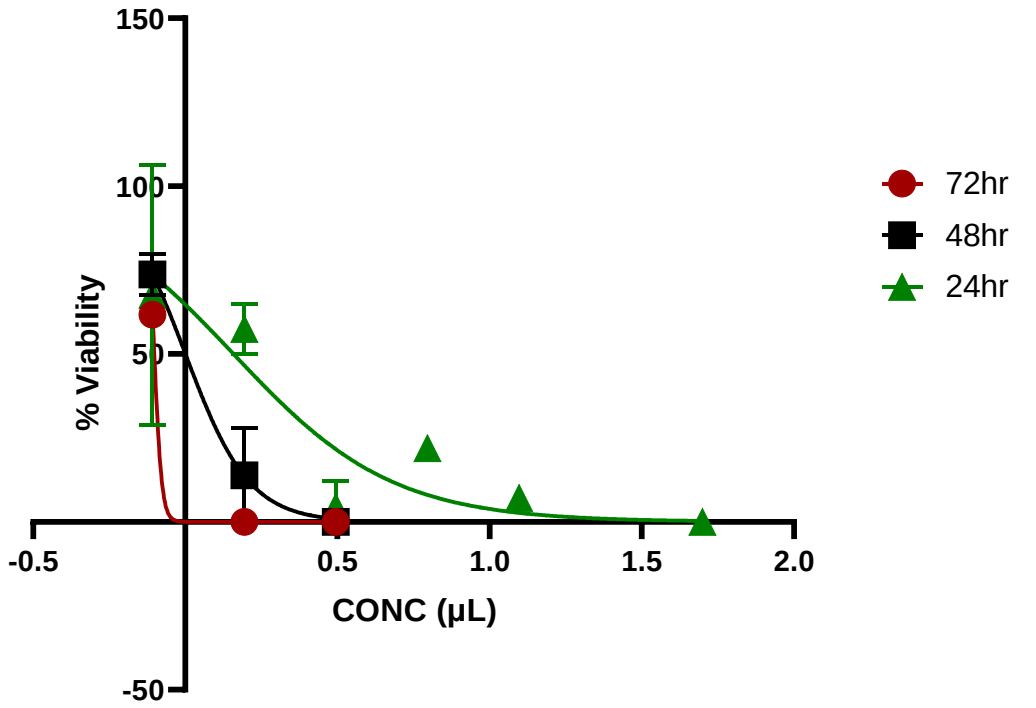

Figure 1: HepG2 IC_{50} on different treatment time (24, 48 and 72 hr).

Table 1: HepG2 IC_{50} on different treatment time (24, 48 and 72 hr) and there R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	1.704	1.332	0.7879
R squared	0.7091	0.9670	0.999

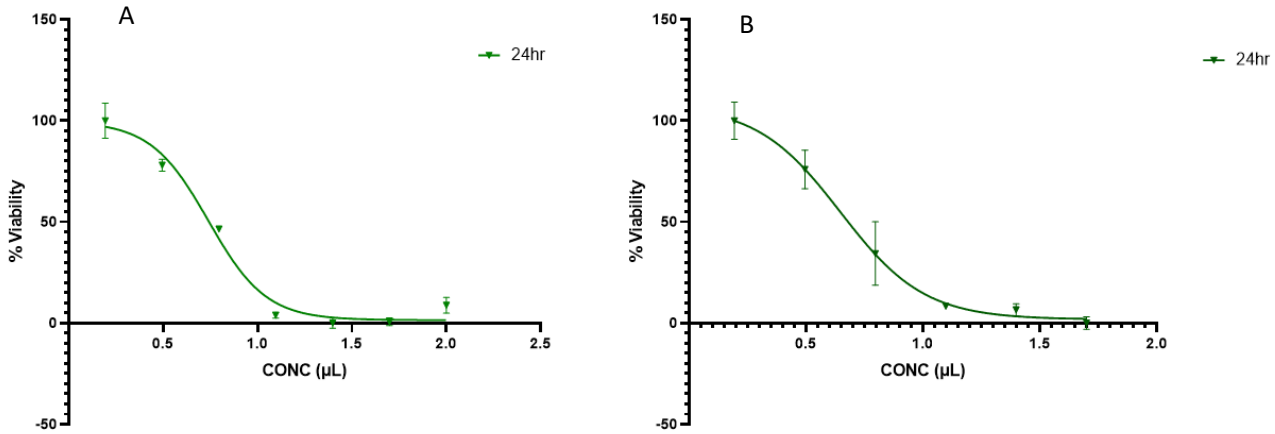

Figure 2: HepG2 IC_{50} trials. A: trial two, B: trial three.

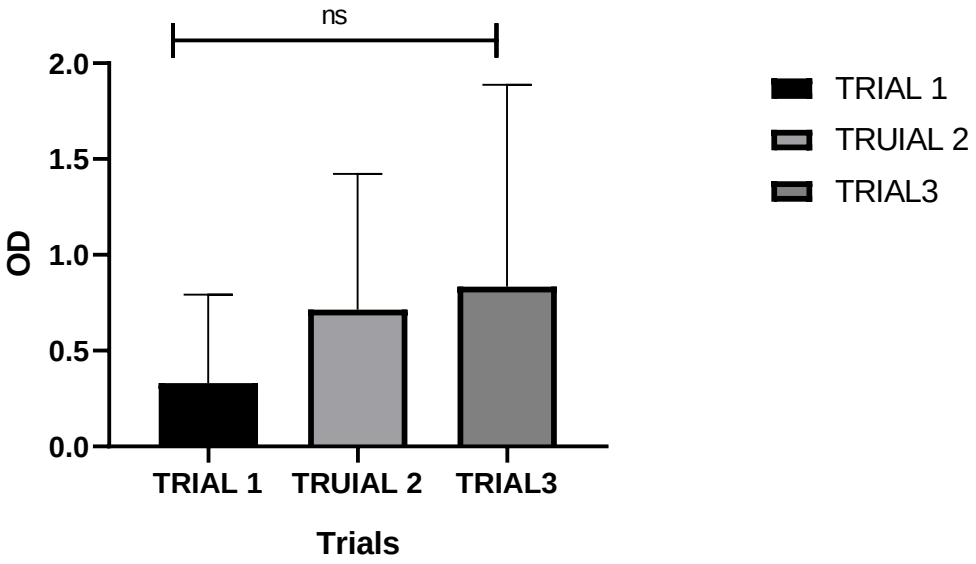

Figure 3: Significant difference between three trials on HepG2 cell line.

3.2 PANC cell line

Epithelioid carcinoma attached cell line that is currently used as an in vitro model to study pancreatic ductal adenocarcinoma carcinogenesis and tumor therapies.

The cytotoxicity of oily extract sample was investigated on different type of cancer and hybrid cell lines. As shown in figure 4 and table 2. In cancerous pancreatic cell line PANC; the half-maximal inhibitory concentration (IC_{50}) value at 24, 48 and 72 hr was 4.96, 5.02 and 3.76 respectively.

The best treatment time was 24 hr so the trial was repeated twice (figure 6). And the result show that; there was no significant difference between the three trails. The IC_{50} for the second and the third trial was 4.56 µl and 6.83 µl; respectively (figure 5).

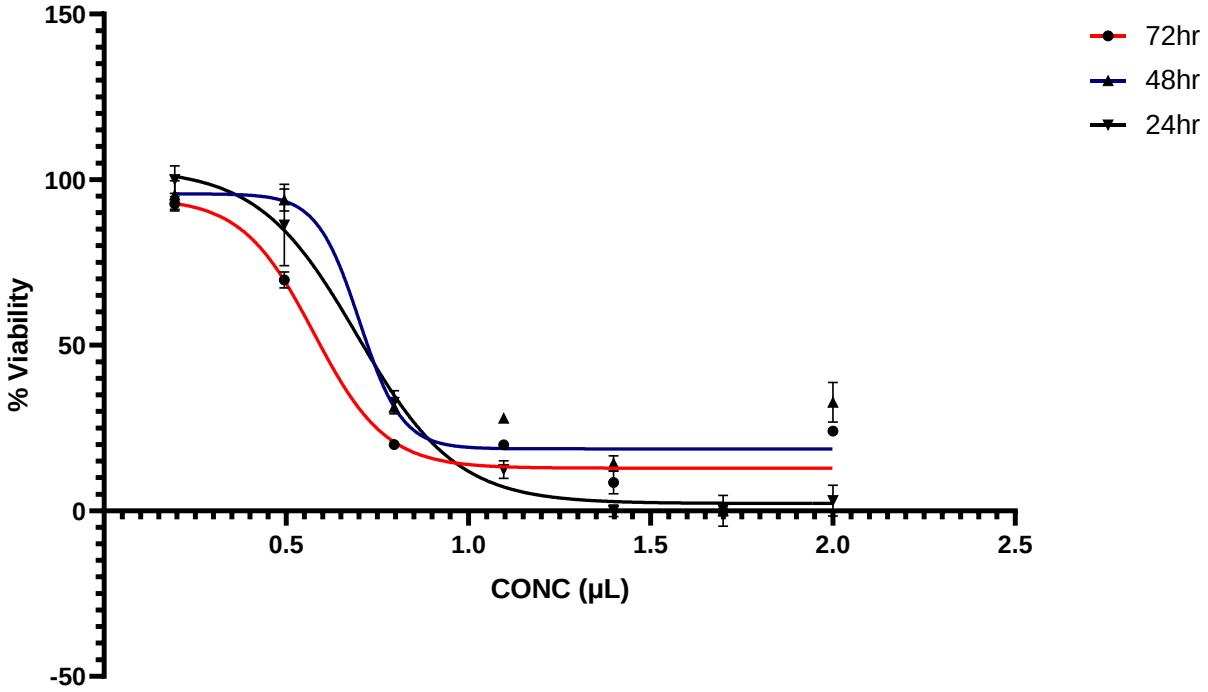

Figure 4: PANC IC_{50} on different treatment time (24, 48 and 72 hr).

Table 2: PANC IC_{50} on different treatment time (24, 48 and 72 hr) and there R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	4.960	5.023	3.764
R squared (R^2)	0.9827	0.9162	0.9471

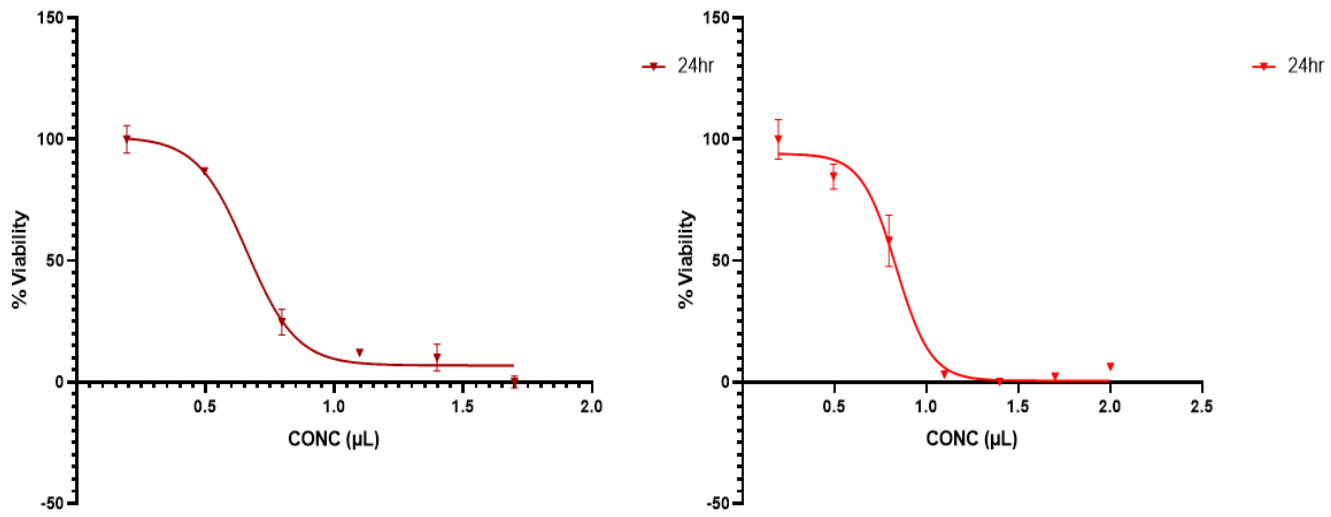

Figure 5: PANC IC_{50} trials. A: trial two, B: trial three.

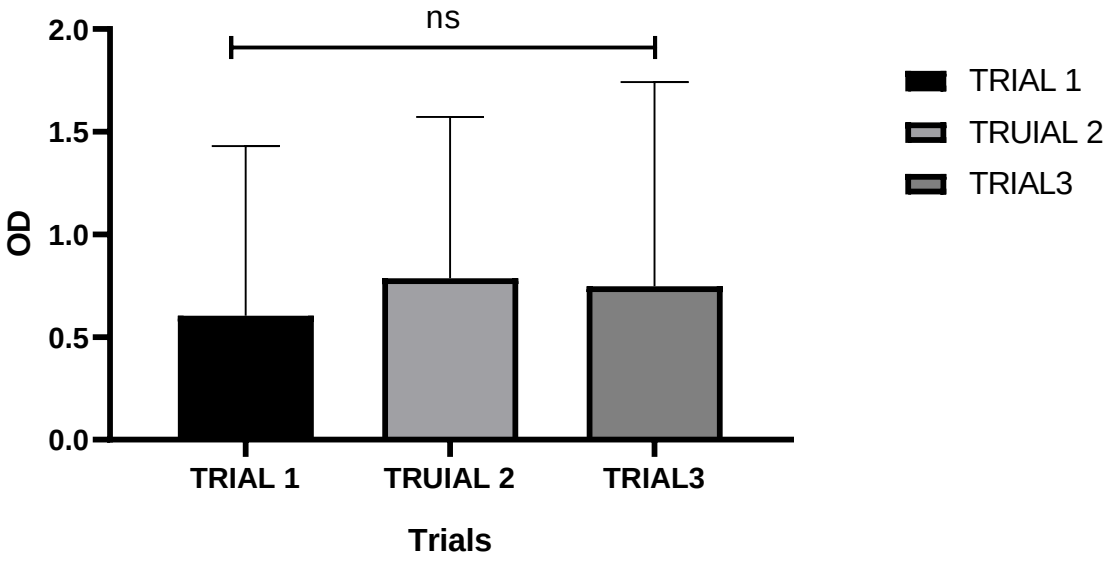

Figure 6: Significant difference between three trials on PANC-1 cell line.

3.3 MIA PACA-2 cell line

The cytotoxicity of oily extract sample was also investigated on epithelial cell line that was derived from tumor tissue of the pancreas MIA PACA-2 cell line. The result showed that; the half-maximal inhibitory concentration (IC_{50}) value at 24, 48 and 72 hr was 7.026, 3.066 and 1.711 respectively (figure 7, table 3).

The best treatment time was 24 hr so the trial was repeated twice. And the result show that; there was no significant difference between the three trails MIA PACA-2 IC_{50} on different treatment time (figure 9). The IC_{50} for the second and the third trial was 12.46 µl and 5.845µl; respectively (figure 8).

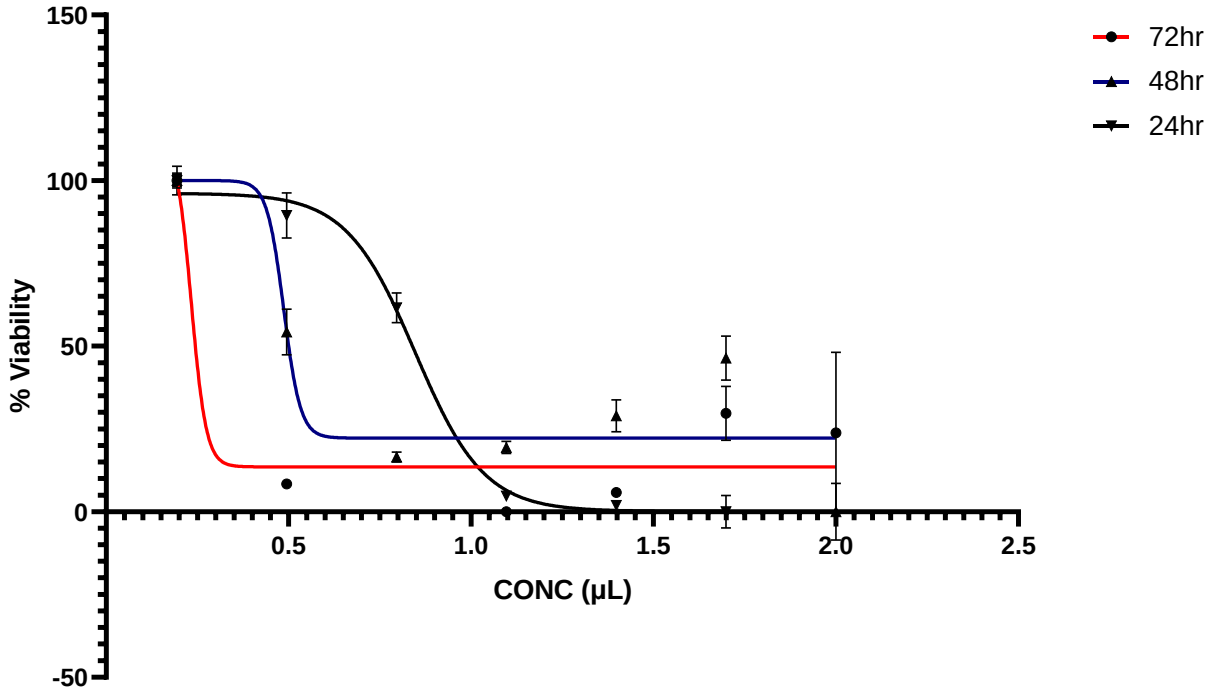

Figure 7: MIA PACA-2 IC_{50} on different treatment time (24, 48 and 72 hr).

Table 3: MIA PACA-2 IC_{50} on different treatment time and R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	7.026	3.066	1.711
R squared (R^2)	0.9894	0.8051	0.8523

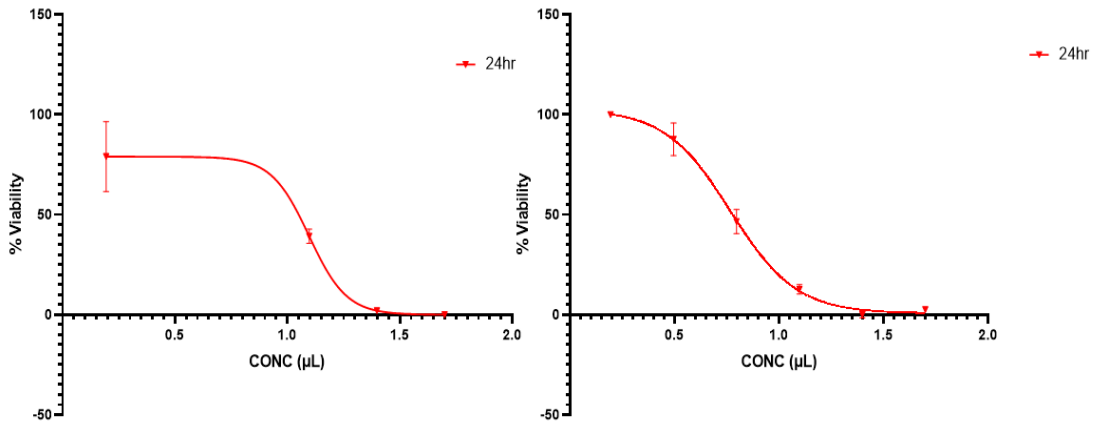

Figure 8: MIA PACA-2 IC_{50} trials. A: trial two, B: trial three.

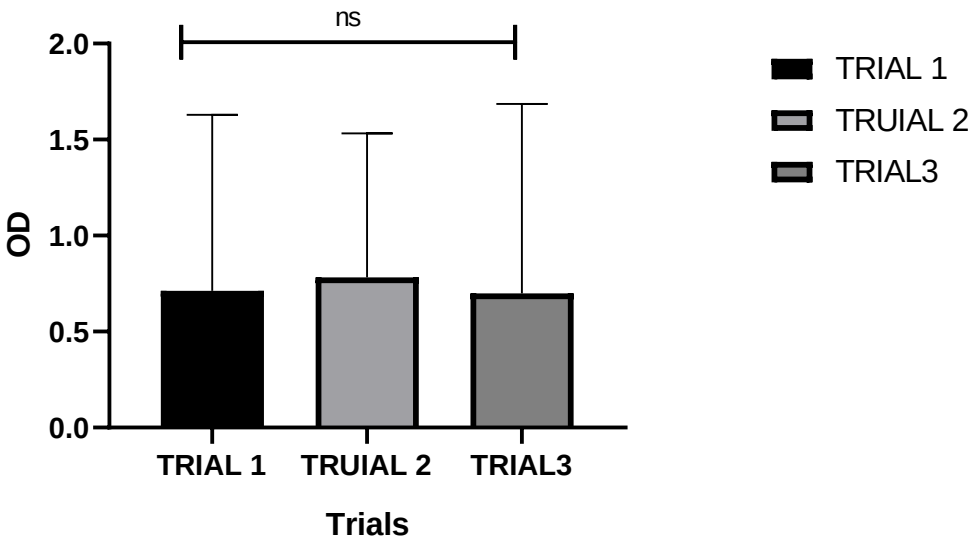

Figure 9: Significant difference between three trials on MIA PACA-2 cell line.

3.4 EA.HY926 cell line

The cytotoxicity of oily extract sample was also investigated on somatic hybrid cell that can be used for cardiovascular disease research EA.HY926 cell line. The result showed that; the half-maximal inhibitory concentration (IC_{50}) value at 24, 48 and 72 hr was 3.042, 2.279 and 3.038 respectively (figure 10, table 4).

The best treatment time was 24 hr so the trial was repeated twice. And the result show that; there was no significant difference between the three trails EA.HY926 IC_{50} on different treatment time (figure 12). The IC_{50} for the second and the third trial was 6.362µl and 7.936 µl; respectively (figure 11).

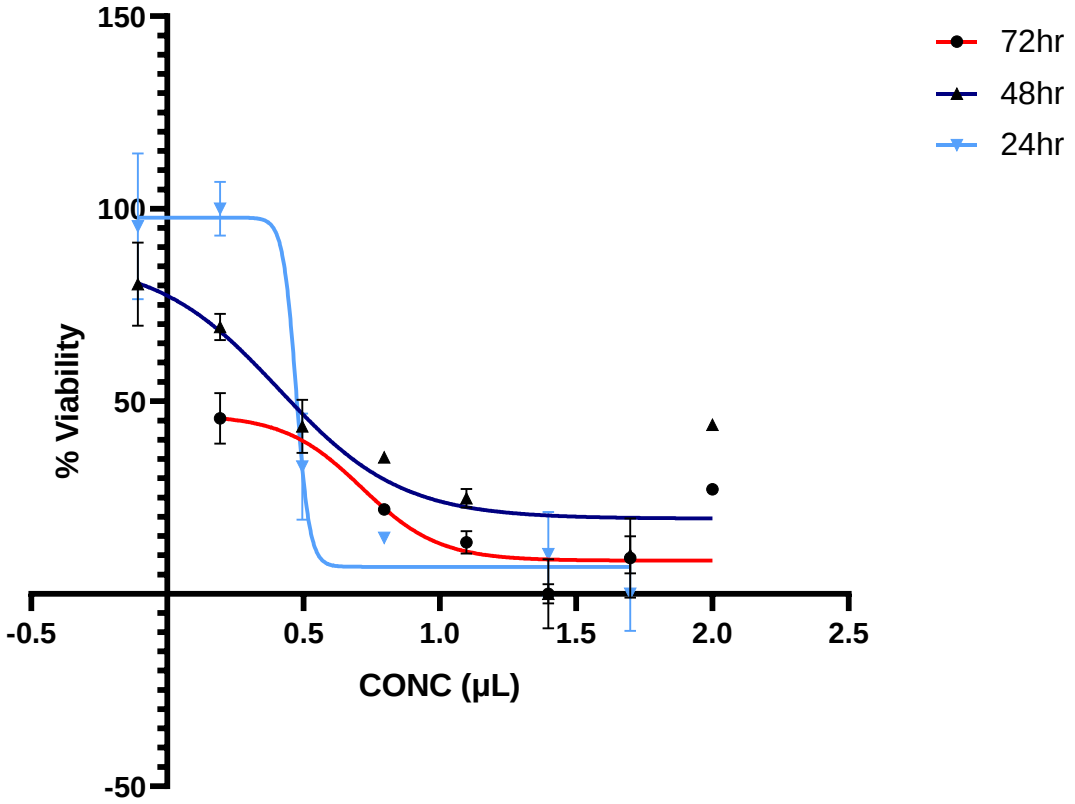

Figure 10: EA.HY926 IC_{50} on different treatment time (24, 48 and 72 hr).

Table 4: EA.HY926 IC_{50} on different treatment time and R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	3.042	2.279	3.038
R squared (R^2)	0.9425	0.7608	0.7396

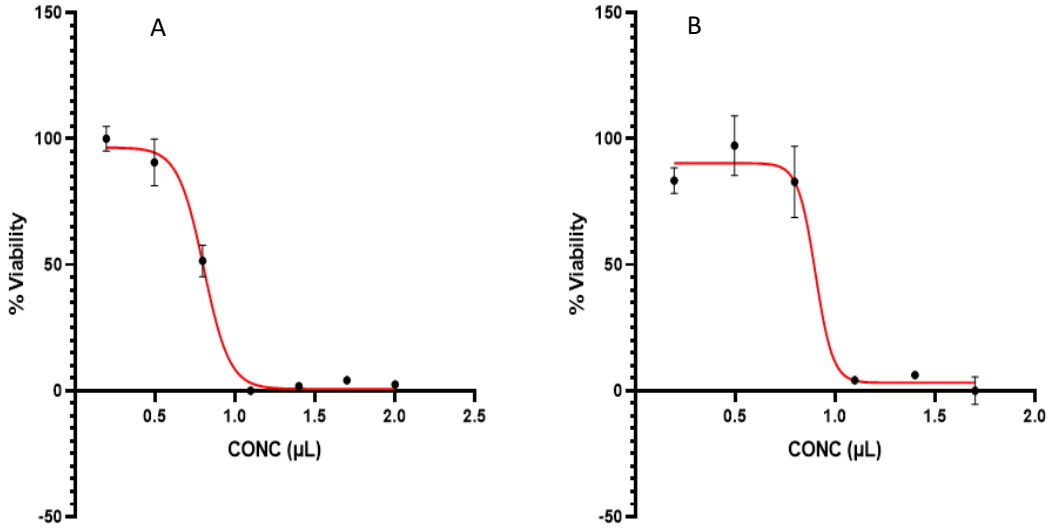

Figure 11: EA.HY926 IC_{50} trials. A: trial two, B: trial three.

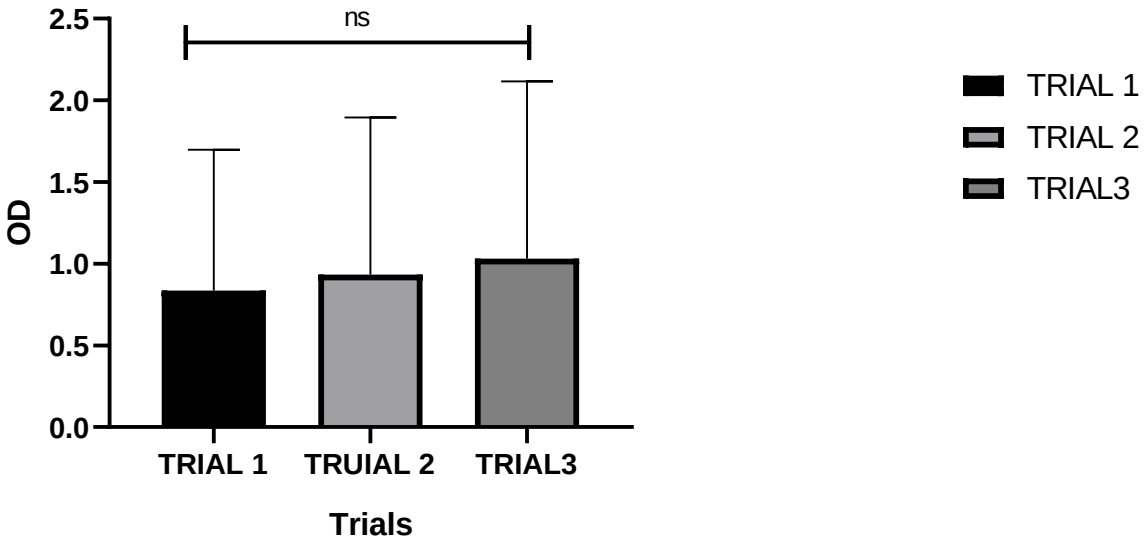

Figure 12: Significant difference between three trials on EA.HY926 cell line.

3.5 MCF7 cell line

Derived from the pleural effusion from a 69 year old female has from a breast adenocarcinoma. It was named after the Michigan Cancer Foundation (MCF) human breast cancer cell line.

The cytotoxicity of oily extract sample was also investigated on MCF7 cell line. As shown in figure 13 and table 5; the have maximal inhibitory concentration (IC50) value at 24 hr,48 and 72 hr was 3.864, 3.291 and 2.646 respectively.

The best treatment time was 24 hr so the trial was repeated twice (figure 15). And the result show that; there was no significant difference between the three trials. The IC50 for the second and third trial was 2.109µl and 3.563µl; respectively (figure 14).

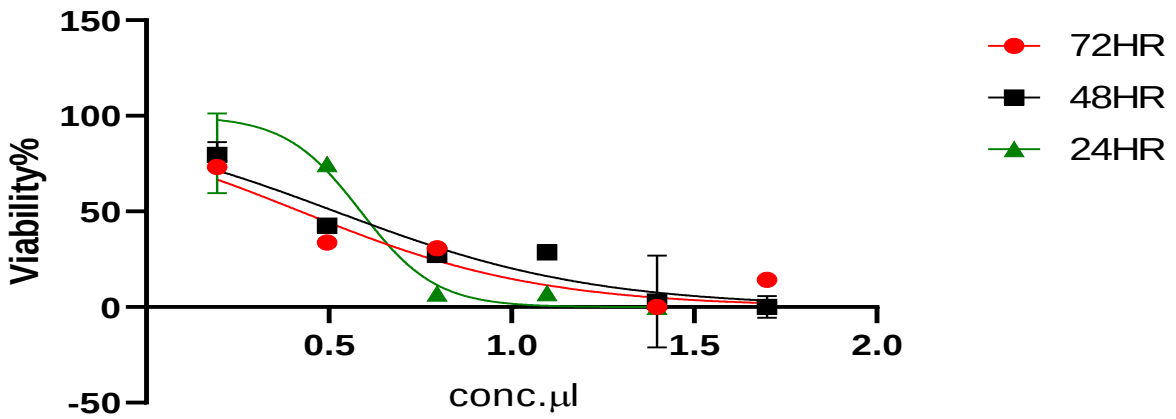

Figure 13: MCF7 IC50 on different treatment time (24,48 and 72 hr).

Table 5: MCF7 IC50 on different treatment time (24,48 and 72 hr) and there R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	3.864	3.291	2.646
R squared	0.8973	0.8044	0.8724

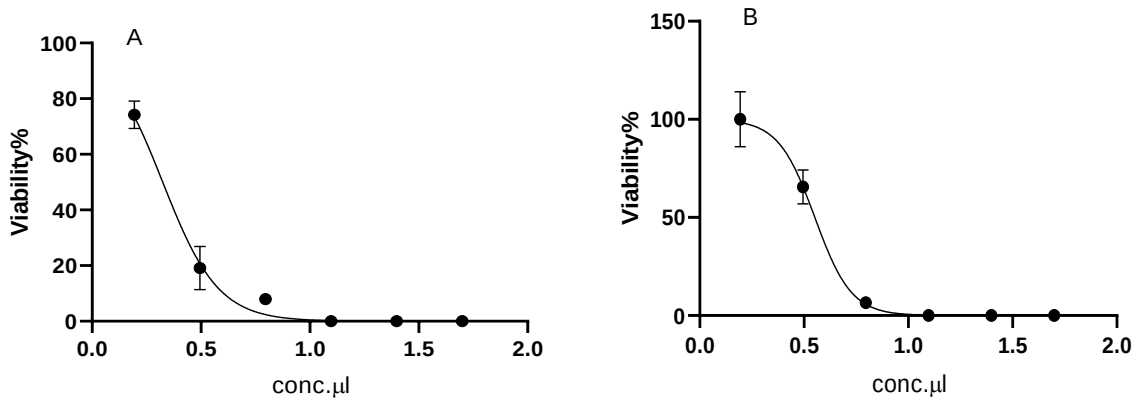

Figure 14: MCF7 IC50 trials. A: trial two, B: trial three.

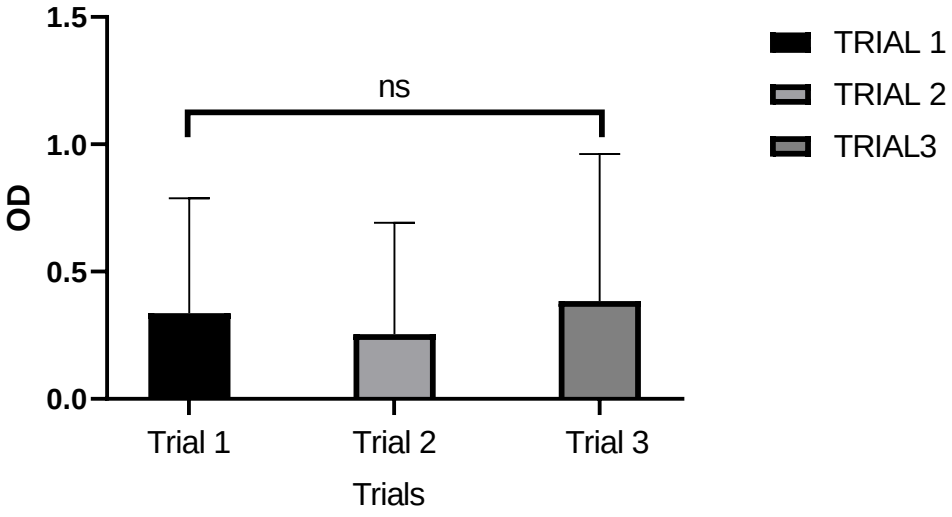

Figure 15: Significant difference between three trials on MCF7 cell line.

3.6 MDA-MB 231 cell line

Derived from the pleural effusion from a 51-year-old caucasian female with a metastatic mammary adenocarcinoma It was an epithelial, human breast cancer cell line.

The cytotoxicity of oily extract sample was also investigated on MDA-MB 231 cell line. As shown in figure 16 and table 6; the have maximal inhibitory concentration (IC50) value at 24 hr,48 and 72 hr was 16.1, 4.477 and 4.453 respectivily.

The best treatment time was 24 hr so the trial was repeated twice (figure 18). And the result show that; there was no significant difference between the three trials. The IC50 for the second and third trial was 5.489µl and 2.928µl; respectively (figure 17).

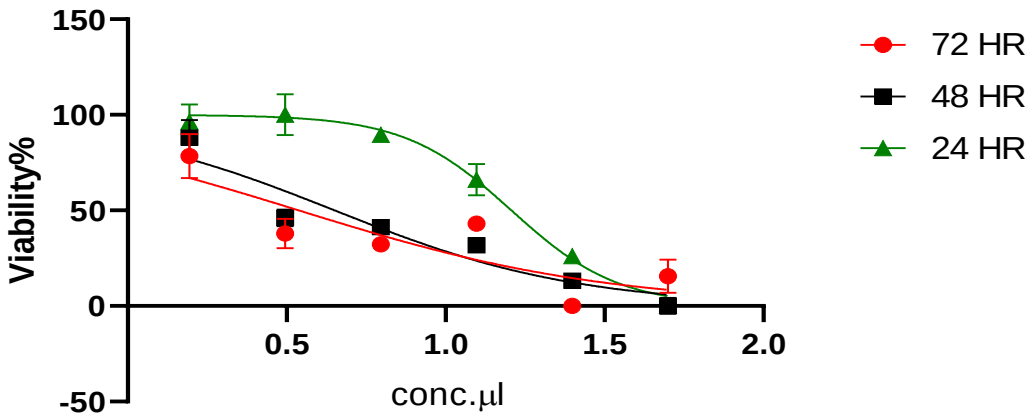

Figure 16: MDA-MB 231 IC50 on different treatment time (24,48 and 72 hr).

Table 6: MDA-MB 231 IC50 on different treatment time (24,48 and 72 hr) and there R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	16.10	4.477	3.453
R squared	0.9733	0.8914	0.6891

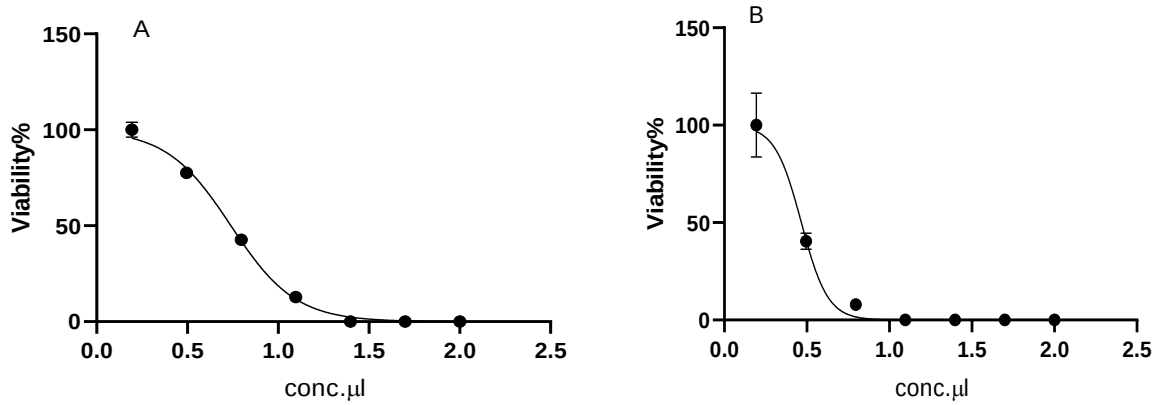

Figure 17: MDA-MB 231 IC50 trials. A: trial two, B: trial three.

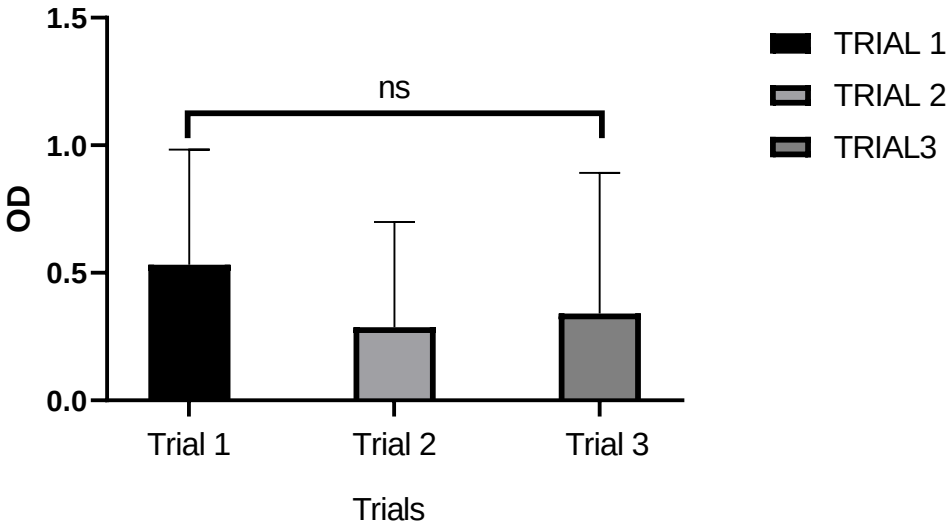

Figure 18: Significant difference between three trials on MDA-MB 231 cell line.

3.7 A549 cell line

Derived from a 58 year old Caucasian male from a type II pneumocyte human lung tumor cancer cell line.

The cytotoxicity of oily extract sample was also investigated on A549 cell line. As shown in figure 19 and table 7; the have maximal inhibitory concentration (IC50) value at 24 hr,48 and 72 hr was 5.401, 5.796 and 4.468 respectively.

The best treatment time was 24 hr so the trial was repeated twice (figure 21). And the result show that; there was no significant difference between the three trials. The IC50 for the second and third trial was 2.646µl and 2.25µl; respectively (figure 20).

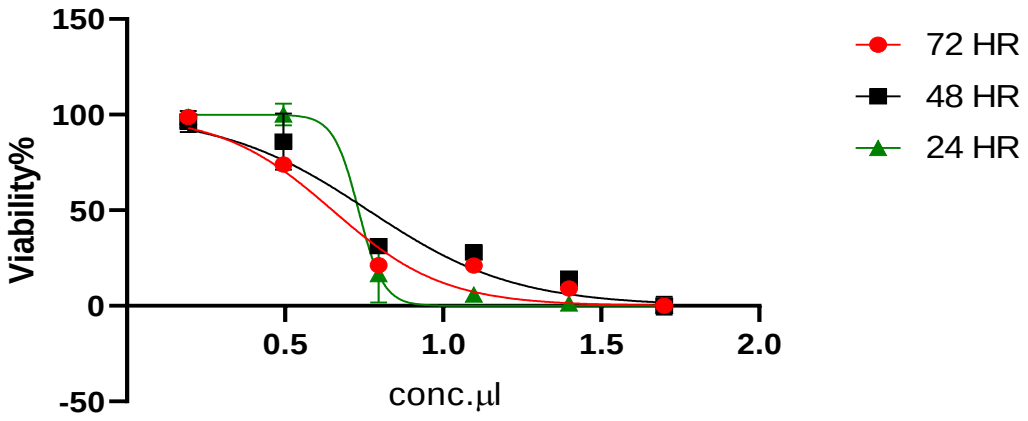

Figure 19: A549 IC50 on different treatment time (24,48 and 72 hr).

Table 7: A549 IC50 on different treatment time (24,48 and 72 hr) and there R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	5.401	5.796	4.468
R squared	0.9811	0.9037	0.9411

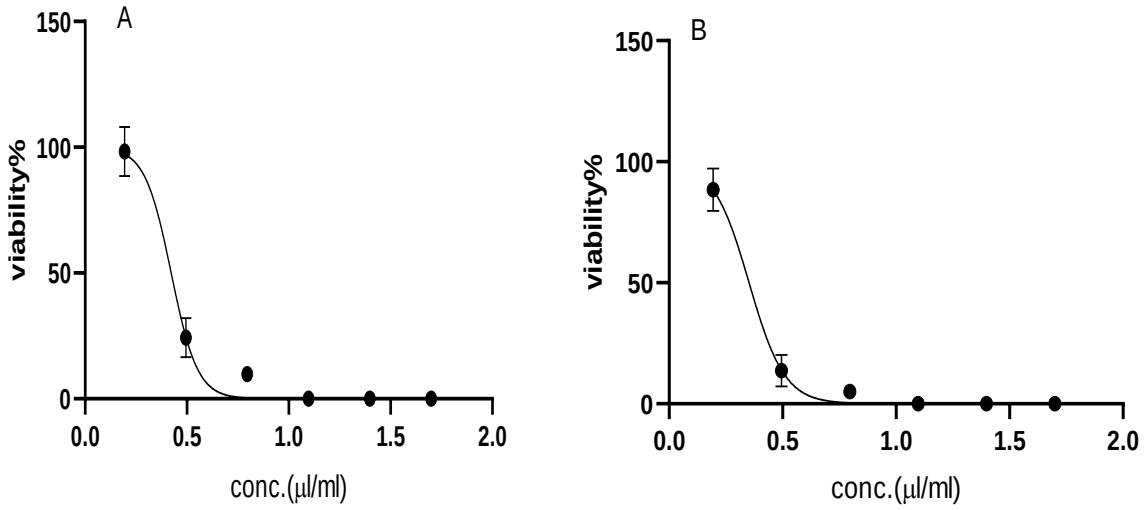

Figure 20: A549 IC50 trials. A: trial two, B: trial three.

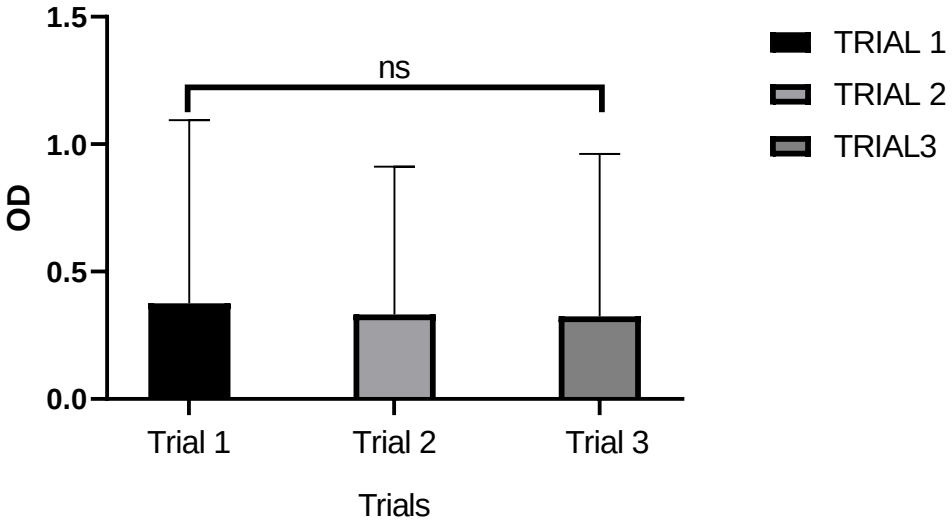

Figure 21: Significant difference between three trials on A549 cell line.

3.8 HT-29 cell line

A primary tumor of a 44 years old Caucasian female from human colon cancers cell line.

The cytotoxicity of oily extract sample was also investigated on HT-29 cell line. As shown in figure 22 and table 8; the have maximal inhibitory concentration (IC50) value at 24 hr,48 and 72 hr was 3.643, 4.101 and 2.7 respectively.

The best treatment time was 24 hr so the trial was repeated twice (figure 24). And the result show that; there was no significant difference between the three trials. The IC50 for the second and third trial was 3.557µl and 2.417µl; respectively (figure 23).

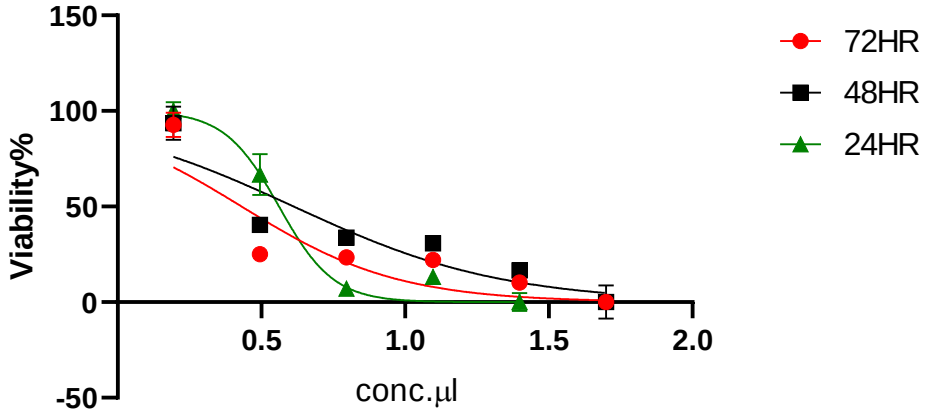

Figure 22: HT-29 IC50 on different treatment time (24,48 and 72 hr).

Table 8: HT-29 IC50 on different treatment time (24,48 and 72 hr) and there R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	3.643	4.101	2.700
R squared	0.9633	0.8253	0.7492

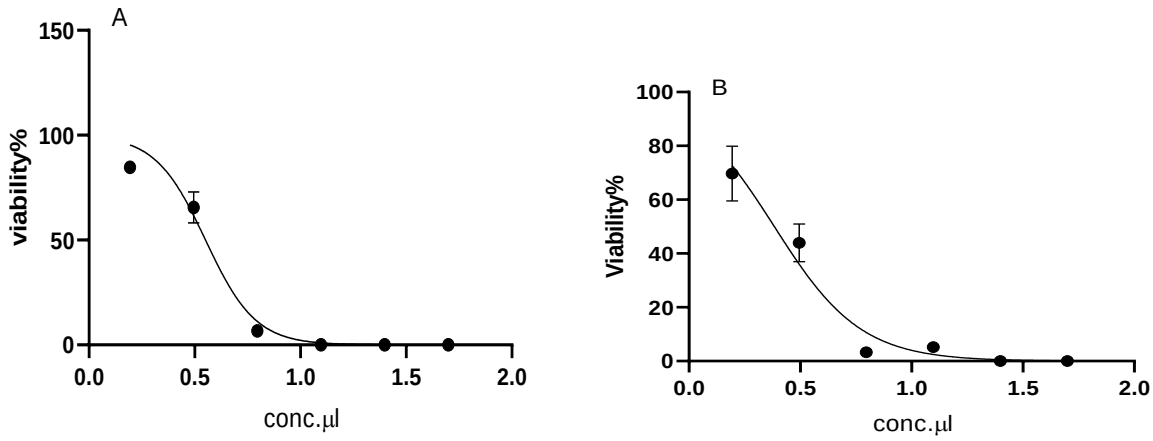

Figure 23: HT-29 IC50 trials. A: trial two, B: trial three.

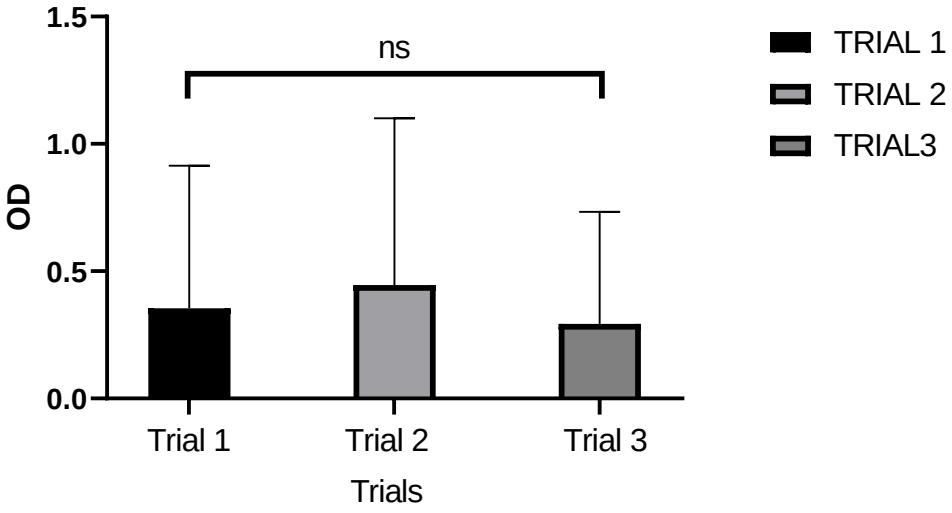

Figure 24: Significant difference between three trials on HT-29 cell line.

3.9 K562 cell line

Derived from a 53-year-old chronic myelogenous leukemia patient.

The cytotoxicity of oily extract sample was also investigated on K562 cell line. As shown in figure 25 and table 9; the have maximal inhibitory concentration (IC50) value at 24 hr,48 and 72 hr was 14.49, 0.7858 and 0.9025 respectively.

The best treatment time was 24 hr so the trial was repeated twice (figure 27). And the result show that; there was no significant difference between the three trials. The IC50 for the second and third trial was 4.609µl and 3.737µl; respectively (figure 26).

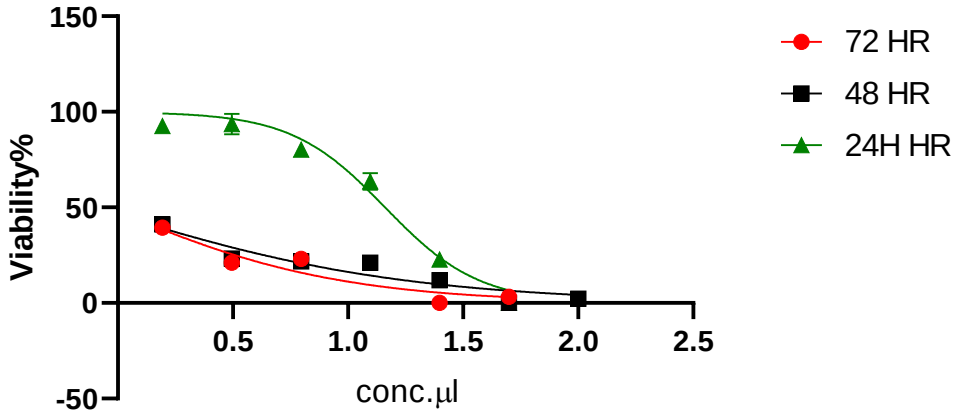

Figure 22: K562 IC50 on different treatment time (24,48 and 72 hr).

Table 9: K562 IC50 on different treatment time (24,48 and 72 hr) and there R^2.

Treatment Time	24 hr	48 hr	72 hr
IC50	14.49	0.7858	0.9025
R squared	0.9735	0.8679	0.8746

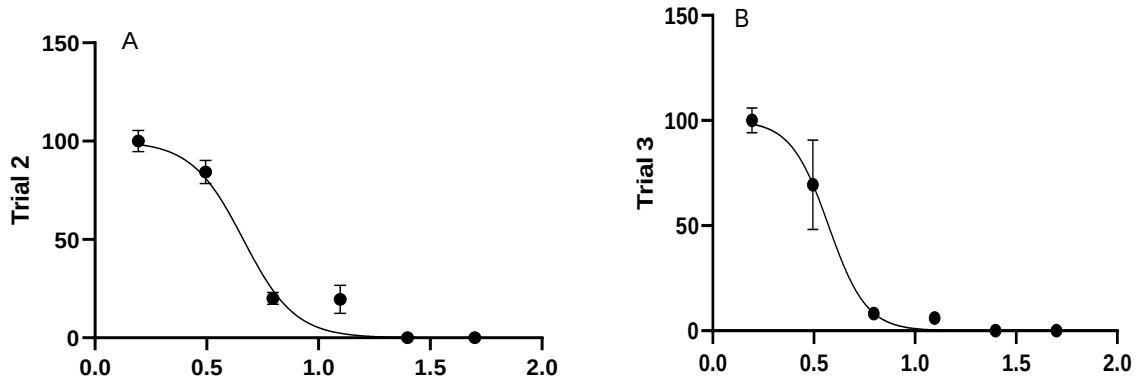

Figure 23: K562 IC50 trials. A: trial two, B: trial three.

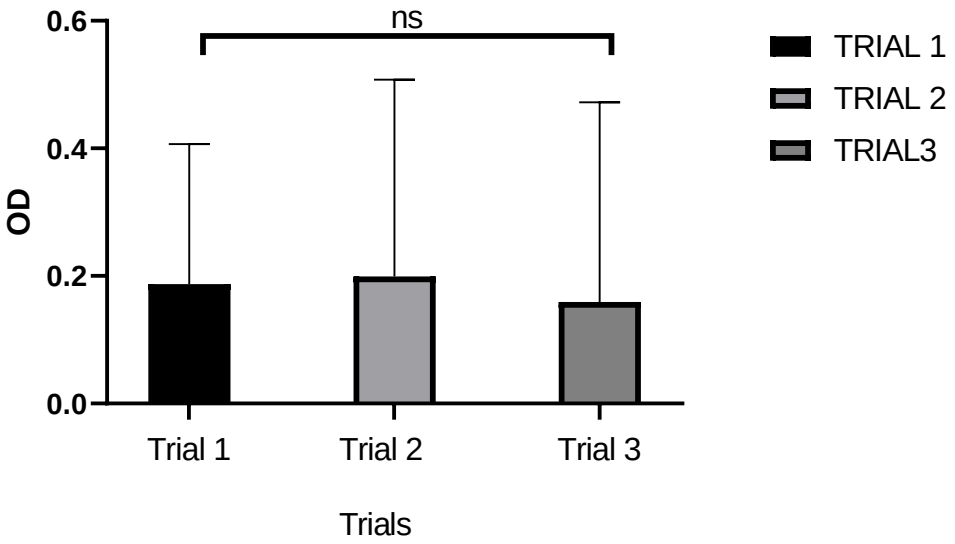

Figure 24: Significant difference between three trials on K562 cell line.

4 Conclusion

The oil exhibits profoundly toxic effects in all examined cell lines, indicating its significant potential as a supplement in the fight against cancer.