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EVALUATION OF ANTI CANCER ACTIVITY AND COMPARITIVE STUDY OF PROBIOTICS AND INDIAN SPICES MIX AGAINST A549 LUNG CANCER CELL LINE

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ABSTRACT

Probiotics are live microorganisms (bacteria, yeast) that provide health benefits when being consumed. These microorganisms are associated with the benefits for humans in reducing the potential of carcinogenic cells. Indian medicinal spices have been used for thousands of years and are known for their vital role in prevention and treatment of cancer. Such traditional Indian herbs used to treat cancer are considerably cheap and have been used to treat types of cancer efficiently. Cancer is the world's biggest human killer disease and it is very complicated to cure because the tumor cells grow abnormal and get metastasized to distinct regions. Tremendous amount of research has been done with the goal of developing better treatments and potential cures for cancer.

Keywords: Probiotics, Medicinal plants, Anticancer activity, A549 Lung Cancer, Tumor cells

INTRODUCTION

Cancer is among the leading causes of morbidity and mortality worldwide. Current therapy available for cancer treatment is associated with number of side effects (Aruna k et al, 1992). However, plants offer alternative route for the treatment of cancer (Jemal et al,2010). Lung cancer is one of the most common cancer types and large number of people dies of this disease every year. In India approximately 63,000 new lung cancers reported every year (Cragg GM, et al, 2000). A549 lung adenocarcinoma cell line comes under the non – small cell lung cancer (NSCLC). It is a heterogeneous group comprised of mostly squamous cell carcinoma, adenocarcinoma and large cell carcinoma (Kapadia GJ et al,1996). Naturally occurring antioxidants give benefits of low toxicity easy bioavailability and possibly enhanced benefits from longer use including anti mutagenic agents (Chen JW et al, 2011). The antioxidants from spices suchas turmeric were experimentally evidenced to control the cellular oxidative stress due

to their antioxidant properties and their capacity to block the production of reactive oxygen radicals and interfering with the signal transduction pathways (Sethi T, 20002). Tannins, saponins , flavonoids, anthraquinones, steroids and glycosides distribution in four medicinal plants belonging to different families were investigated and compared (Saxelin M,et al 2005). Qualitative phytochemical analysis is found out by using protocol proposed by (Vejselova D et al, 2015) The presence of eight major secondary metabolites such as proteins, tannins, saponins, flavonoids, steroids , carbohydrates ,anthraquinone and alkaloids was carried out(Divisi D, et al, 2006). The antioxidant properties of medicinal herbs were studied using Trolox equivalent antioxidant capacity, DPPH scavenging and reducing power and total polyphenol contents by (Gupta VK,2006). The antimicrobial screening of some Indian spices and their resistance over various microbes by various methods(Aggarwal BB , et al ,2006). The relation between Diet and cancer by intake of healthy antioxidant rich foods for the prevention of cancer (Mohammad S, 2006). Systematic screening of total antioxidants in dietary plants and spices where in the potential of dietary plants against cancer with immense antioxidants was experimented (Mensor L.L et al,2001)

MATERIALS AND METHODS

SAMPLE COLLECTION

The three common spices pepper, turmeric and cinnamon each of 50gm were collected. They were dried and powdered. The samples were meshed thoroughly to get the fine powdery texture and was stored for the further consecutive analysis.

EXTRACTION OF SAMPLES

Soxhlet method:

The sample was washed with distilled water to remove any adherent particles, shade dried and powdered. 25g of sample was weighed and extracted with 300 ml of ethanol by continuous hot percolation with the help of soxhlet apparatus for 10hrs of time. On completion the extract were filtered and concentrated using rotary evaporator under reduced pressure and controlled temperature of 50ºc - 60ºc.The concentrates were stored in the refrigerator for further use. The same procedure was used for each of three samples of pepper, cinnamon and turmeric for extraction.

QUALITATIVE ANALYSIS

Phytochemical Test:

The filtered crude extracts of all three spices was subjected for phytochemical screening for the identification of presence or absence of secondary metabolites in every spice sample collected and extracted. The screening was performed for tannins, saponins, flavonoids, alkaloids, proteins and phenols. The colour intensity or the precipitate formation was used as analytical responses to these tests.

Antioxidant assay:

Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8 ml of absolute methanol was added to blank. 100 ml of BHT was added and 100 µl of respective samples to all other tubes marked as tests. 200 µl of DPPH reagent was added to all the test tubes including blank. All the test tubes were incubated at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

Calculation:

$$\%\text{Antioxidant activity} = \frac{(\text{absorbance at blank}) - (\text{absorbance at test}) \times 100}{(\text{absorbance at blank})}$$

Cell proliferation assay:

The cells were plated in 24 well plates and incubated in 37ºc with 5% co2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/ml (5mg/ml) of 0.5% 3-(4,5 –dimethyl – 2- thiazolyl)-2,5 diphenyl- tetrazolium bromide (MTT) was added and incubated for 4 hrs. After incubation ,1 ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with UV – spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC 50) was determined graphically.

Cell lines and cell cultures:

A549 cell line was obtained from NCCS pune. The cells were maintained in minimal essential medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO2 at 37ºc.

RESULTS AND DISCUSSION

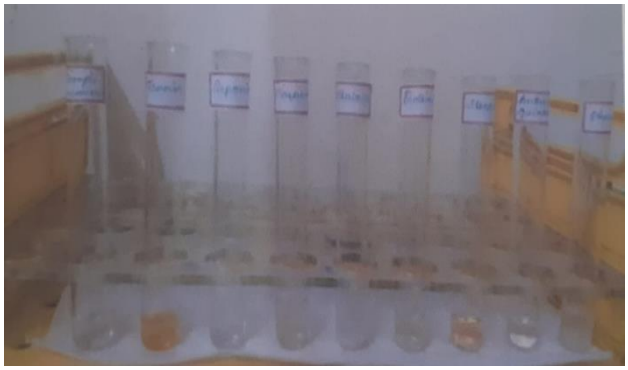
SOLVENT EXTRACTION

The extract were filtered and concentrated using rotary evaporator under reduced pressure and controlled temperature of 50°C -60°C.The concentrates were stored in the refrigerator for further use.

Representative image of the extracted spice samples by soxhlet method

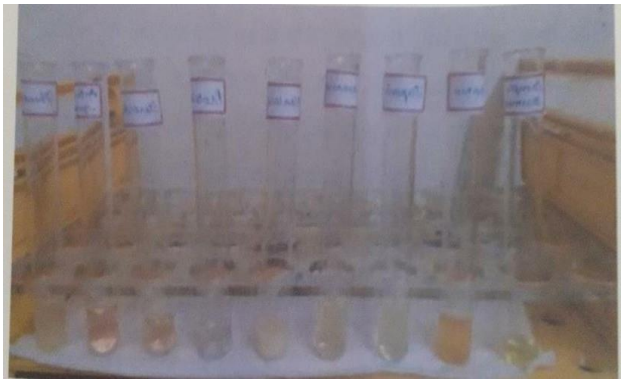
PHYTOCHEMICAL ANALYSIS

Phytochemical analysis to find the presence of the active secondary metabolites in all the three samples was experimented individually.



Representative image depicts the phytochemical test of cinnamon

Representative image depicts the phytochemical test of Pepper



Representative image depicts the phytochemical test of Turmeric

Table1: Presence and absence of secondary metabolites in Cinnamon

TEST	PRESENCE/ABSENCE
TANNINS	+
SAPONINS	+
FLAVONOIDS	+
ALKALOIDS	-
PROTEINS	+
STEROIDS	+
ANTHRAQUINONE	-
PHENOL	+

Table2: Presence and absence of secondary metabolites in Pepper

TEST	PRESENCE/ABSENCE
TANNINS	+
SAPONINS	+
FLAVONOIDS	-
ALKALOIDS	-
PROTEIN	-
STEROIDS	+
ANTHRAQUINONE	-
PHENOL	+

Table3: Presence and absence of secondary metabolites in Turmeric

TEST	PRESENCE / ABSENCE
TANNINS	-
SAPONINS	+
FLAVONOIDS	+
ALKALOIDS	-
PROTEIN	-
STEROIDS	+
ANTHRAQUINONE	+
PHENOL	+

ANTIOXIDANT ASSAY

The antioxidant activity of given samples was obtained using DPPH assay method for three spices extract and the spice with the higher antioxidant activity was found.

Table 4: OD & DPPH values of three spices

S.NO	SAMPLE	CONCENTRATION (µg/ml)	O.D	DPPH ACTIVITY (%)
1	Cinnamon	1000	0.305	60.44
2	Pepper	1000	0.387	49.80
3	turmeric	1000	0.336	56.42

BLANK OD: 0.771

The above table depicts the results obtained for three different spice samples by DPPH assay. The test samples were taken in concentration 1000µg/ ml. The highest % DPPH activity among the three species was shown by cinnamon. The least % DPPH activity was shown by pepper amongst the other species .Thus the cinnamon has high antioxidant activity amongst other two species.

MTT ASSAY:

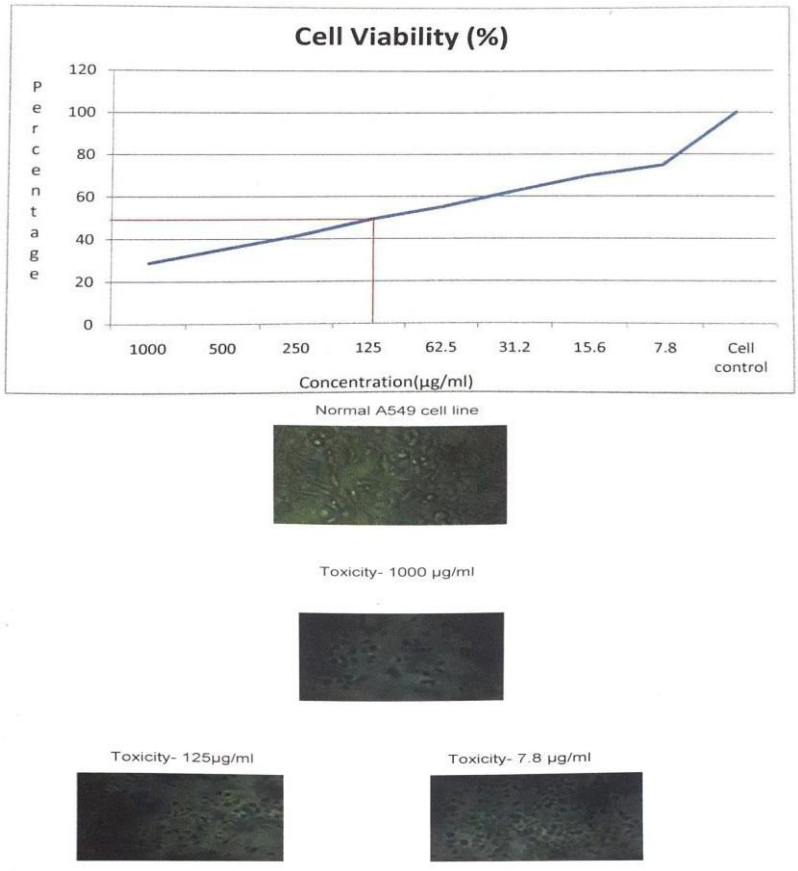
The in vitro cell proliferation of the test samples against the A549 cell lines were assessed through MTT assay method, and this was carried out in three phases separately.

$$\% \text{ cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

Table 5: Anticancer effect of probiotics on A549 cell line

S.NO	Conc(µg/ml)	Dilutions	Absorbance (O.D)	Cell viability(%)
1	1000	neat	0.515	28.72
2	500	1:1	0.632	35.24
3	250	1:2	0.743	41.43
4	125	1:4	0.879	49.02
5	62.5	1:8	0.986	54.91
6	31.2	1:16	1.12	62.46
7	15.6	1:32	1.25	69.71
8	7.8	1:64	1.34	74.73
9	Cell control	-	1.793	100

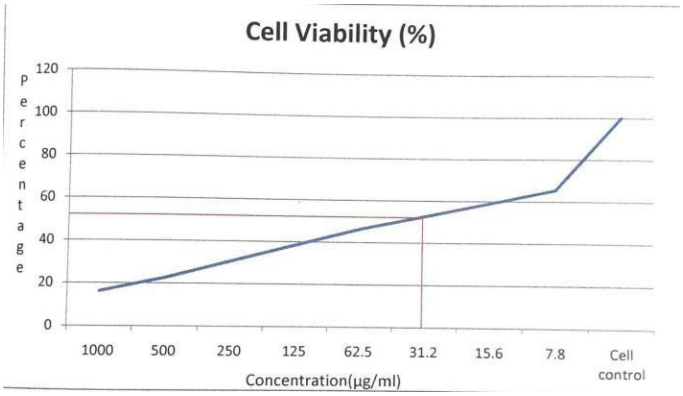
Graphical representation of anticancer activity of Probiotics against A549 lung cancer cell lines.



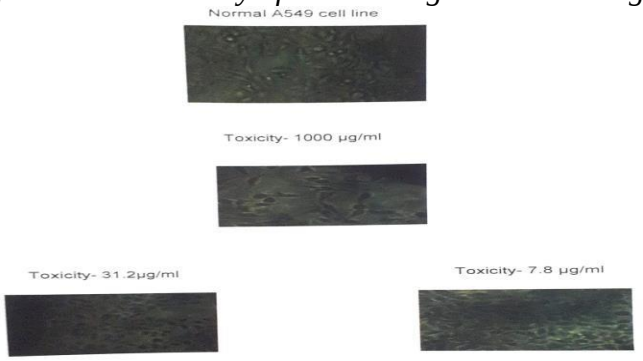
The above picture depicts the growth arrest of the A549 lung cancer cell lines at different concentrations of Probiotics sample.

Table 6: Anticancer effect of turmeric on A549 cell line

S.No	Conc(µg/ml)	Dilution	Absorbance	Cell viability(%)
1	1000	neat	0.277	16.17
2	500	1:1	0.386	22.53
3	250	1:2	0.524	3.058
4	125	1:4	0.660	38.52
5	62.5	1:8	0.793	46.29
6	31.2	1:16	0.901	52.59
7	15.6	1:32	1.010	58.96
8	7.8	1:64	1.12	65.38
9	Cell control	-	1.713	100



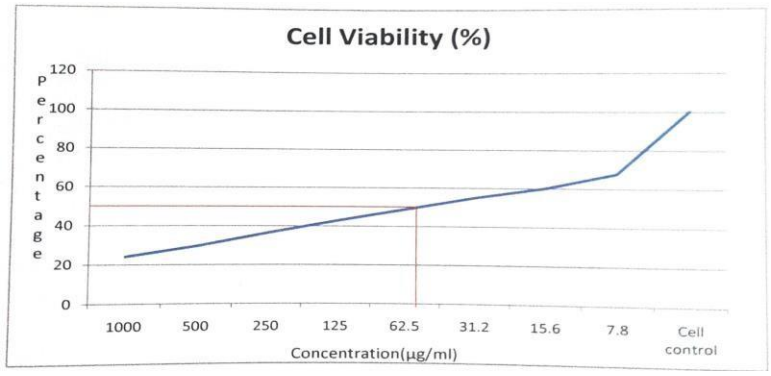
Graphical representation of anticancer activity of turmeric against A549 lung cancer cell lines.



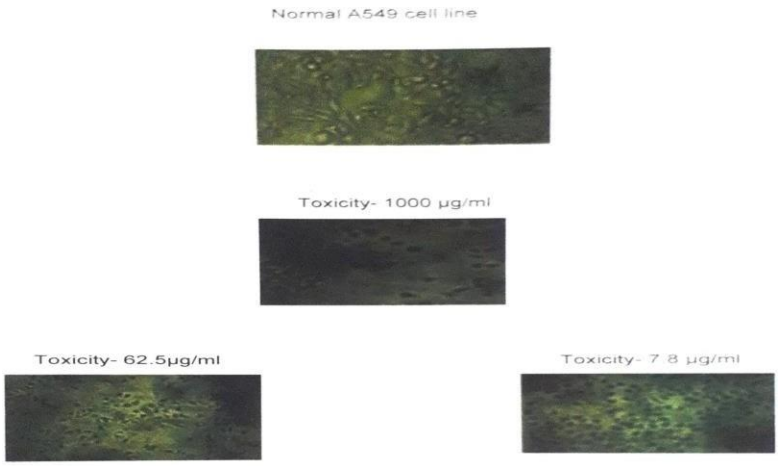
The above picture depicts the growth arrest of the A549 lung cancer cell lines at different concentrations of Turmeric sample.

Table 7: Anticancer effect of pepper on A549 cell line

S.NO	Conc(µg/ml)	Dilution	Absorbance	Cell viability (%)
1	1000	Neat	0.412	24.05
2	500	1:1	0.506	29.53
3	250	1:2	0.622	36.31
4	125	1:4	0.731	42.67
5	62.5	1:8	0.836	48.80
6	31.2	1:16	0.941	54.93
7	15.6	1:32	1.03	60.12
8	7.8	1:64	1.16	67.71
9	Cell control	-	1.713	100



Graphical representation of anticancer activity of Pepper against A549 lung cancer cell lines

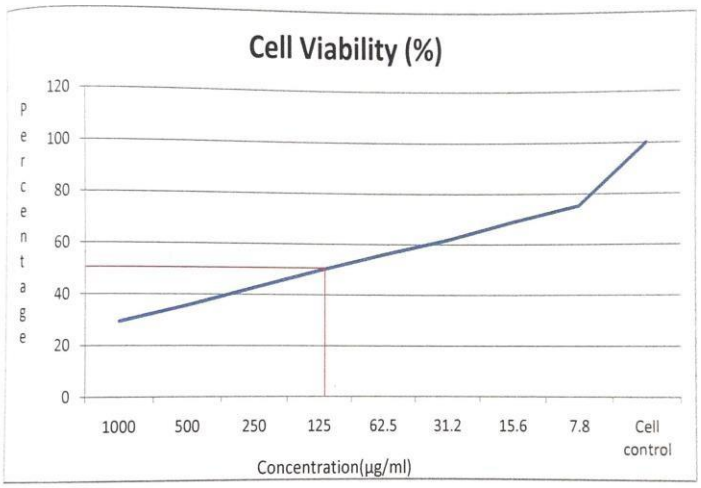


The above picture depicts the growth arrest of the A549 lung cancer cell lines at different concentrations of Pepper sample.

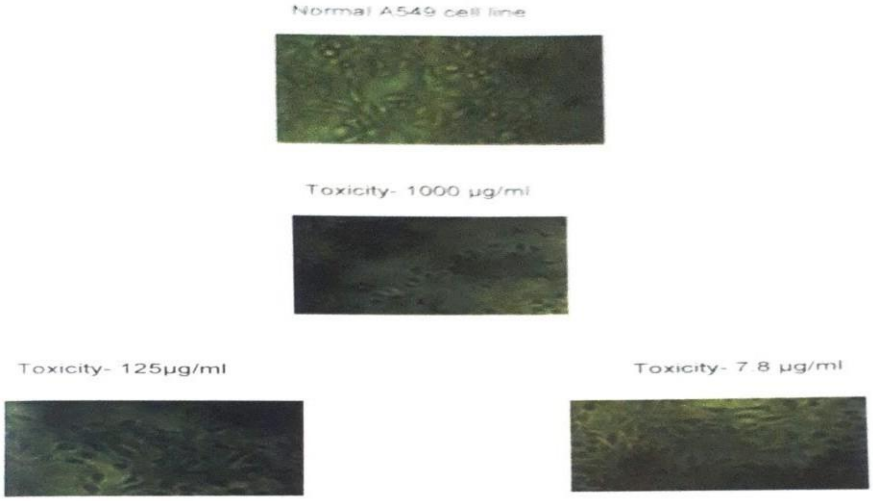
Table 8: Anticancer effect of cinnamon on A549 cell line

S.NO	Conc(µg/ml)	Dilution	Absorbance	Cell viability (%)
1	1000	Neat	0.503	29.36
2	500	1:1	0.612	35.72
3	250	1:2	0.733	42.79
4	125	1:4	0.851	49.67
5	62.5	1:8	0.959	55.98

6	31.2	1:16	1.06	61.87
7	15.6	1:32	1.18	68.88
8	7.8	1:64	1.29	75.30
9	Cell control	-	1.713	100



Graphical representation of anticancer activity of Cinnamon against A549 lung cancer cell lines

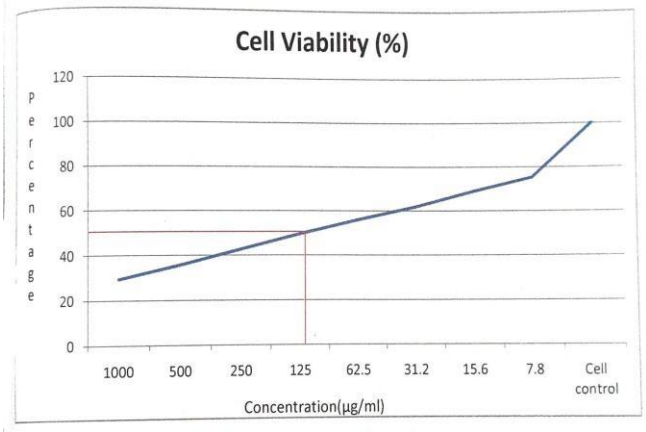


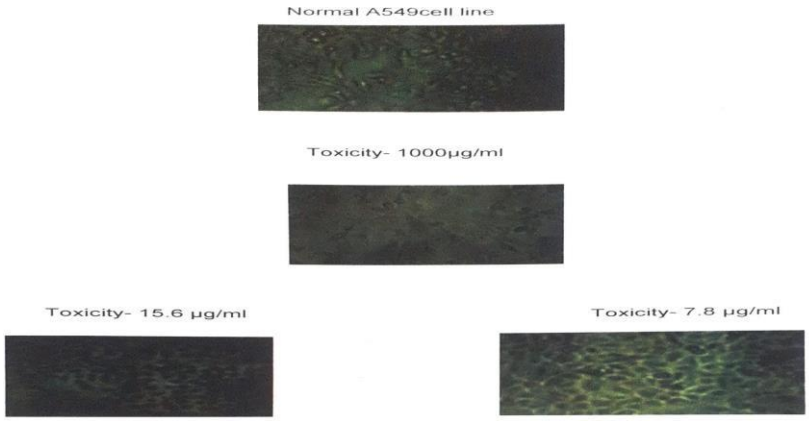
The above picture depicts the growth arrest of the A549 lung cancer cell lines at different concentrations of Cinnamon sample.

Table 9: Anticancer effect of turmeric and probiotic on A549 cell line

S.NO	Conc(µg/ml)	Dilution	Absorbance	Cell viability(%)
1	1000	Neat	0.288	16.83
2	500	1:1	0.367	21.44
3	250	1:2	0.469	27.41
4	125	1:4	0.566	33.08
5	62.5	1:8	0.674	39.39
6	31.2	1:16	0.786	45.93
7	15.6	1:32	0.877	51.25
8	7.8	1:64	0.989	57.80
9	Cell control	-	1.711	100

Graphical representation of anticancer activity of turmeric and probiotic against A549 lung cancer cell lines

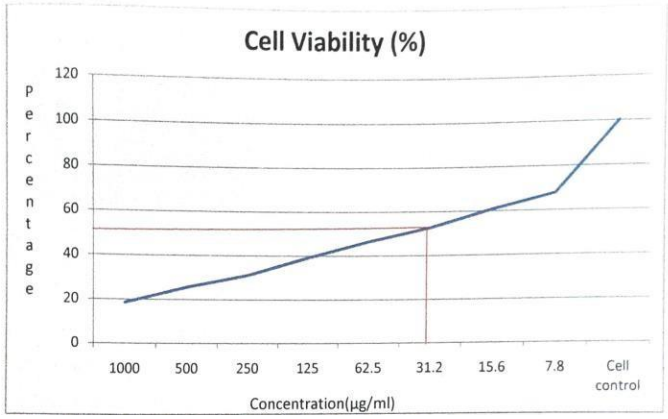




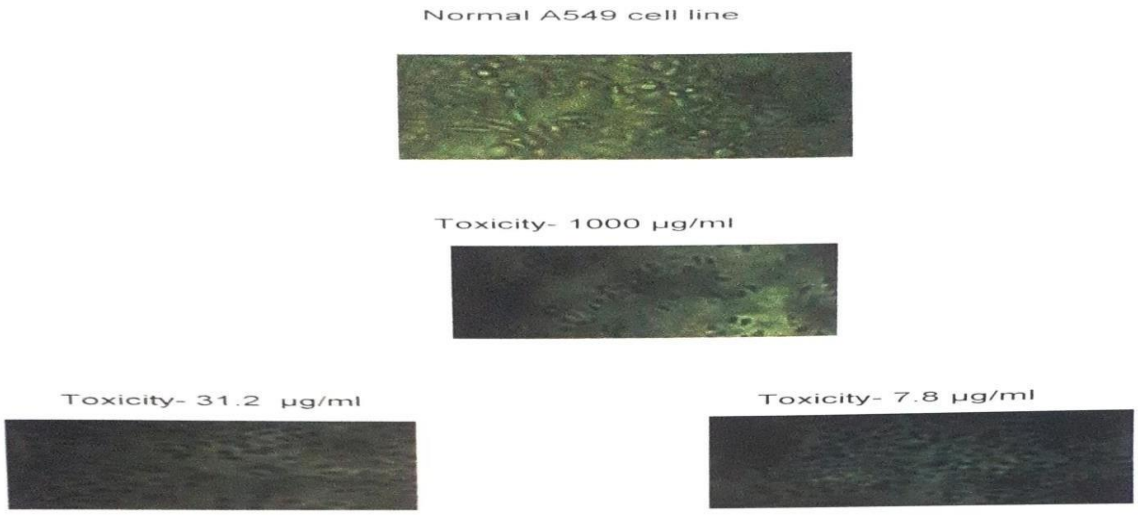
The above picture depicts the growth arrest of the A549 lung cancer cell lines by the turmeric and probiotic at different concentrations (µg/ml).

Table 10: Anticancer effect of pepper and probiotic on A549 cell line

S.NO	Conc(µg/ml)	Dilution	Absorbance	Cell viability (%)
1	1000	Neat	0.16	18.46
2	500	1:1	0.436	25.48
3	250	1:2	0.532	31.09
4	125	1:4	0.671	39.21
5	62.5	1:8	0.796	46.52
6	31.2	1:16	0.902	52.71
7	15.6	1:32	1.04	60.78
8	7.8	1:64	1.16	67.79
9	Cell control	-	1.711	100



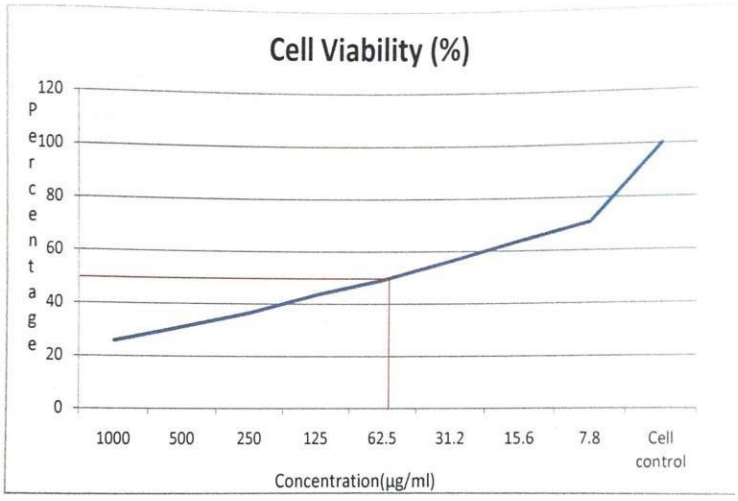
Graphical representation of anticancer activity of Pepper and probiotic against A549 lung cancer cell lines



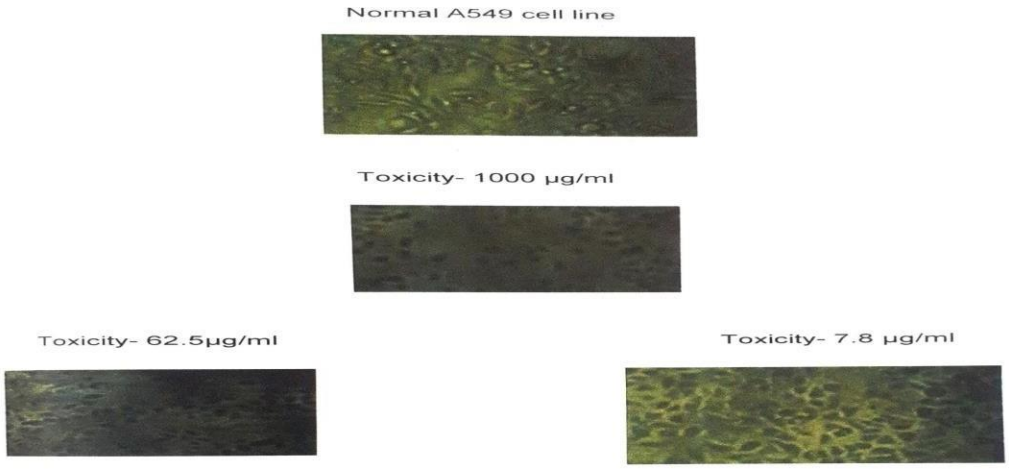
The above picture depicts the growth arrest of the A549 lung cancer cell lines by the Pepper and probiotic at different concentrations (µg/ml).

Table 11: Anticancer effect of cinnamon and probiotic on A549 cell line

S.NO	Conc(µg/ml)	Dilution	Absorbance	Cell viability (%)
1	1000	Neat	0.439	25.65
2	500	1:1	0.532	31.09
3	250	1:2	0.626	36.58
4	125	1:4	0.745	43.54
5	62.5	1:8	0.841	49.15
6	31.2	1:16	0.964	56.34
7	15.6	1:32	1.09	63.70
8	7.8	1:64	1.21	70.71
9	Cell control	-	1.711	100



\ Graphical representation of anticancer activity of Cinnamon and probiotic against A549 lung cancer cell lines



The above picture depicts the growth arrest of the A549 lung cancer cell lines by the Cinnamon and probiotic at different concentrations (µg/ml).

The anticancer activity was experimented against A549 LUNG CARCINOMA cell lines with all the three samples and probiotics. The % cell viability of each test samples was calculated and the graph was plotted. From the study and the obtained values, it was understood that Turmeric (Curcuma longa) shows the best anticancer effect with the less % cell viability lung carcinoma cell lines both individually (16.17) and in combination with probiotics (16.87) was found to be least value that exhibits the growth arrest of the Cancer cell lines at the concentration of 1000µg/ml.

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