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# IN VITRO CYTOTOXIC ACTIVITY OF AQUEOUS EXTRACT OF SEEDS OF *OPUNTIA FICUS-INDICA* PLANT AGAINST HELA CANCER CELL LINE

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**ABSTRACT**

Research is focusing on the search for new types of natural chemotherapeutic agents derived from plants which are proving to be excellent sources of new compounds. The aqueous extract of Opuntia ficus-indica plant was screened for cytotoxic activities by standard MTT and SRB assays against three cancerous viz., A549, HeLa and MCF-7 and a normal HEK293 cell cultures. The successive aqueous extract showed selective toxicity towards cancer cells and showed less toxicity towards normal cells. The successive aqueous fraction showed potent activity against HeLa cells with $IC_{50}$ 39.717µg/ml and 85.63 µg/ml by MTT and SRB assay methods respectively and was comparatively nontoxic towards normal HEK293 cell cultures. The aqueous extract had no activity against A549 and MCF-7 cell cultures.

**Key words:** Cytotoxicity, SRB, MTT, Extraction.

## INTRODUCTION

Natural products are known to provide lead compounds in the past and play a significant role in future in the treatment of cancer. The Opuntia ficus-indica is found in arid tropics and sub-tropics with winter rains; grown as hedge plant; spineless types are identified; a cactus-fruit, oblong to pear shaped; pulp soft, whitish, translucent with many seeds, juicy and can be eaten fresh or dried; baked fruits are good for whooping cough; raised from cuttings [1]. Cactus pear fruit seeds exhibit considerable variations in form, size, structure, embryo characteristics, and testa color. They represent about 10-15% of the edible pulp and are usually discarded as waste after pulp extraction. Several authors have reported a great variation in the number of seeds, from 1-5 to more than 2000 per fruit [2-6]. This variation is observed within and/or between species depending on factors such as the age and size of the plant, and the number of flowers per plant. Seed vitality under natural or controlled storage conditions will depend on many factors, including seed type, maturity stage, viability and moisture content during storage, temperature and degree of fungal or bacterial infection. However a systematic study to reveal its cytotoxicity against normal and cancer cells have not undertaken yet. Hence, in the present study we report the cytotoxic activity of the crude extracts from seeds of Opuntia ficus-indica in both normal and cancer cell cultures.

## Materials and methods

### Plant material

Opuntia ficus-indica seeds were obtained from pujan nursery, Ahmedabad, Gujarat. The seeds were washed with sterile water, dried in shade, finely powdered & stored in air tight bottles.

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**Preparation of Plant extract**

10 gm of dried, finely grounded powder of *Opuntia ficus-indica* seeds was immersed in 150 ml of methanol and extracted by reflux extraction at 40 ºC for 3 hours. After extraction, the extract was filtered through Watmann filter paper and evaporated till dryness. 100 Microgram of plant extract was dissolved in the 1 ml DMSO and then 1:3 dilution of test compound was prepared for MTT and SRB assay.

**Phytochemical investigation of *Opuntia ficus-indica* seeds**

**Test for steroids** :Liebermann –Burchard reaction : Mix 2ml extract with chloroform .Add 1-2 ml aceteic anhydride and 2 drops conc. $H_2SO_4$ from the side of test tube. First red, then blue and finally green color appears.

**Test for Saponins**

Foam test: shake the drug extract or dry powder vigorously with water. Persistent foam observed.

**Test for Flavonoids :**

*Shinoda test*: To dry powder or extract, add 5ml 95%ethanol, few drops conc. HCl and .5gms magnesium turnings, Pink color observed.

**Cell culture**

Human embryonic kidney cell line (HEK 293T), lung adeno carcinoma cell line (A549 cell line), cervical cancer cell line (Hela cell line) and breast cancer cell line MCF-7 was grown in DMEM (Dulbecco's modifications of eugal's medium with L-glutamine & 4.5G/L glucose) supplemented with fetal bovin serum 100 units/ml of penicillin G and 0.1 mg/ml of streptomycin sulfate in a humidified atmosphere of a 5% $CO_2$ at 37°C.

**Cytotoxicity assay by MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium Bromide) assay method [7-10]**

The monolayer cell culture was trypsinized and the cell count was adjusted to 3 lac cells/ ml using medium containing 10% newborn calf serum. Pre incubate cells at a concentration of $1\times 10^6$ cells/ml in culture medium for 3 h at 37°C and 6.5% $CO_2$. The cells were seeded at a concentration of $5\times 10^4$ cells/well in 100 µl culture medium and incubated at 37ºC in 5 % $CO_2$ incubator for 24 hrs. After 24 hours, when the monolayer formed, the supernatant was flicked off and added previously diluted with media of 100µl of different concentrations of test extract in microtitre plates and kept for incubation at 37ºC in 5 % $CO_2$ incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. The media was removed after 72 hours and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (10 mL, 5 mg/mL in PBS, pH 7.4) was added followed by 4 hours incubation at 37ºC in 5% $CO_2$ incubator. MTT is reduced by viable cells to a purple formazan product. Following removal of media, the formazan was solubilized by addition of Isopropanol (100 mL) to each well. The absorbance was measured using a microplate reader at 590 nm with a reference filter of 620 nm. The percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:

The percentage growth inhibition was calculated using following formula,

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}} \times 100$$

**Cytotoxicity assay by SRB method [11]**

The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0 x 105 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100µl of different test compound concentrations were added to the cells in microtitre plates. The plates were then incubated at 37oC for 72 hours in 5% CO2 incubator and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25µl of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form a over all concentrations 10%. The plates were incubated at 4oC for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, sample and serum, and were then air-dried. The air dried plates were stained with 100µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100µl of 10mM Tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 540nm.

The percentage growth inhibition was calculated using following formula,

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}} \times 100$$

**Results and Discussion**

***In vitro* cytotoxic activity of aqueous extract of Opuntia *ficus-indica* by MTT assay**

The effect of methotrexate and *Opuntia ficus-indica*, was performed on four different cell lines by MTT assay. Dose response curves constructed for MTT method between the range of 5.0-100000 n gm/ml. Calculation of $IC_{50}$ and $R^2$ values was done using graphs generated from Microsoft excel 2007 edition .The susceptibility of cells to the drug exposure was characterized by $IC_{50}$ values.

Results indicate that the antiproliferative effect strengthens with increase in the concentration of drug.

From table 1, observed that the highest cytotoxic activity was found with methotrexate against A549 and HeLa having $IC_{50}$: 9.70 and 27.90 µ gm/ml respectively. Lower activities were in HEK-293 and MCF-7 having $IC_{50}$:45.42 and 49.42 µ gm/ml.

From figure 1a, it showed Methotrexate has the dose-effect co-relation with maximum linearity in case of A549, MCF-7, HEK 293 and HeLa, respectively with increasing with increase in the concentration & attains linearity till $IC_{50}$ value is reached.

Interestingly, observed that methotraxate showed cytotoxic activity against A549, HeLa, MCF-7 cancer cell lines and also the normal kidney cell line i.e., HEK-293T cell line show better results in terms of $IC_{50}$ and regression due to its antimetabolite activity which inhibit dihydro folate reductase enzyme.

From table 1, observed that cytotoxic activity of aqueous extract of *Opuntia ficus-indica* have found against only HeLa cell line with $IC_{50}$: 39.717µ gm/ml and other cell lines have no activity

From figure 1b, showed that aqueous extract of *Opuntia ficus-indica* has the dose-effect co-relation with maximum linearity in case of HeLa of the four cell lines at value being 0.948.The % inhibition is increasing with increase in the concentration.

The other cell lines show insignificant regression with non linearity in the values of change of % inhibition with the increase in concentration.

After evaluation Out of the four cell lines tested, HeLa only showed results in terms of $IC_{50}$ and regression where other two cancer cell line (A549, MCF-7) and normal kidney cell line (HEK-293) does not reach upto 50% cell growth inhibition and also non linear.

**In *vitro* metabolic assay for *Opuntia ficus-indica* aqueous extract and methotrexate by SRB assay:**

The effect of Methotrexate and *Opuntia ficus-indica* extract was performed on four different cell lines by SRB assay. Dose response curves constructed for SRB method between the range of 5.0-100000 ngm/ml. Calculation of $IC_{50}$ and $R^2$ values was done using graphs generated from Microsoft excel 2007 edition .The susceptibility of cells to the drug exposure was characterized by $IC_{50}$ values. Results indicate that the antiproliferative effect strengthens with increase in the concentration of drug. The $IC_{50}$ values of test extract as well as standard drug (methotrexate) were summarised in table 2 and figure 2(a) & (b).

From table 2, observed that highest activity of Methotrexate have found against HeLa and A549 having $IC_{50}$: 7.7643 and 16.112 respectively. Lower activities were in MCF-7 and HEK 293 ($IC_{50}$: 16.531 and 45.671).

From figure 2a, showed that methotrexate has the dose-effect co-relation with maximum linearity in case of HeLa of the four cell lines at value being 0.9747. The % inhibition is increasing with linearity till $IC_{50}$ value is reached. Linearity in case of A549, HEK 293 and MCF-7 cell lines with increasing with increase in the concentration & attains linearity till $IC_{50}$ value is reached respectively.

So, methotraxate shows cytotoxic activity against A549, HeLa, MCF-7 cancer cell lines and also the normal kidney cell line i.e., HEK-293T cell line show better results in terms of $IC_{50}$ and regression due to its antimetabolite activity which inhibit dihydro folate reductase enzyme

From table 2, observed that cytotoxic activity of Aqueous extract of *Opuntia ficus-indica* have found against only HeLa cell line with $IC_{50}$: 85.63µ gm/ml and other A549, MCF-7 cancerous and HEK 293 normal cell lines have no activity.

From figure 2b, showed that aqueous extract of Opuntia *ficus-indica* has the dose-effect co-relation with maximum linearity in case of HeLa of the four cell lines at value being 0.967.The % inhibition is increasing with increase in the conc. linearly.

The other cell lines A549 and MCF-7 and also normal kidney cell line i.e HEK 293 show insignificant regression with non linearity and % inhibition of also these three cell lines have not reach up to 50% cell growh inhibition with increase in concentration

After evaluation Out of the four cell lines tested, HeLa showed results in terms of $IC_{50}$ and regression.

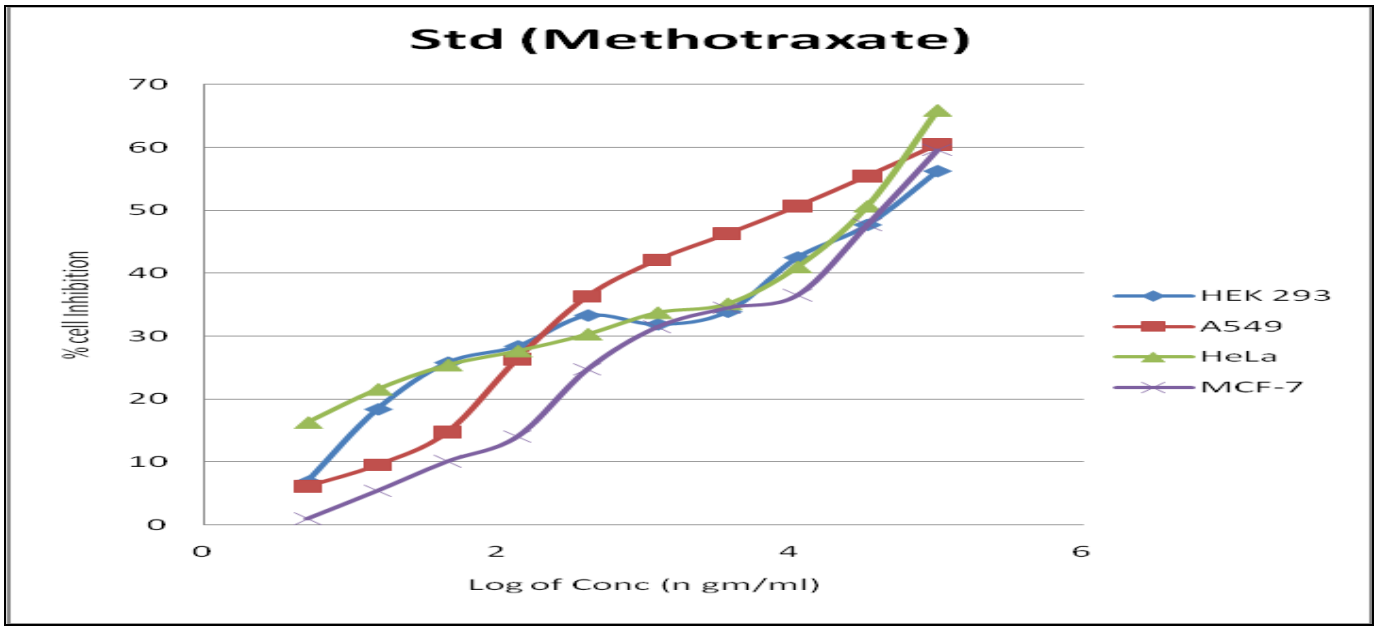
**Discussion**

The results of cytotoxicity study of methanolic extract of *Opuntia ficus-indica* showed significacant cytotoxicity against A549 cell line, whereas this extract had no activity against HeLa, MCF-7 cell lines. This methanolic extract of *Opuntia ficus-indica* is non toxic to normal cells, but showed excellent toxicity on cancer cells. The cytotoxicity of methanolic extract of *Opuntia ficus-indica* may be due to the presence of flavonoids having mono to poly phenolic groups in the structure. The flavonoids have reported for their cytotoxic activity due to presence of phenolic groups [12].

The extractive value, total polyphenolic content and anti cancer activity was at its peak in methanolic extract indicating that most of the active components are extracted with methanol. Cytotoxic changes observed was cell aggregation, cell rounding and cell death. The overall results indicates the promising baseline information for the potential uses of the methanol extracts of tegmen of *Opuntia ficus-indica* seed as an anti cancer agent.

**Figure 1(a) & (b).** % Growth inhibition of methotrexate and acqueous extract of *Opuntia ficus-indica* against HEK 293, A549, HeLa, and MCF-7 by the MTT assay

**A**




**B**

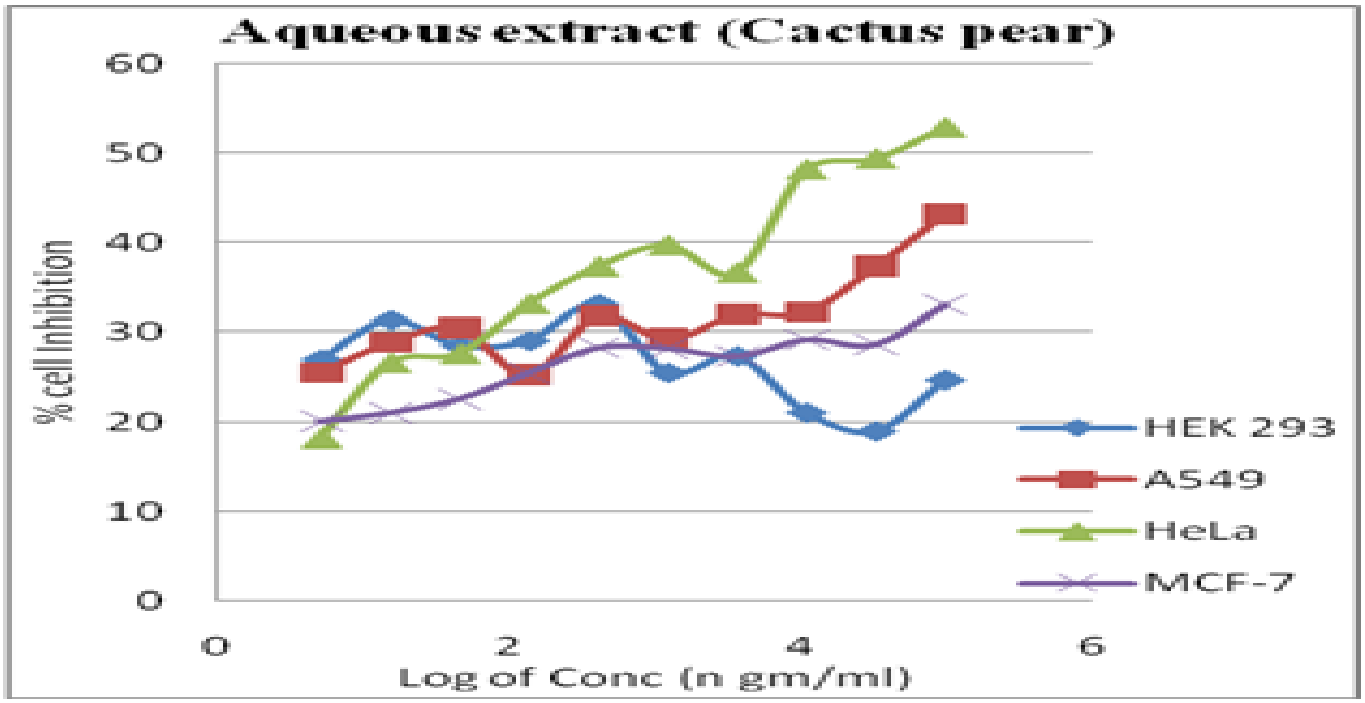
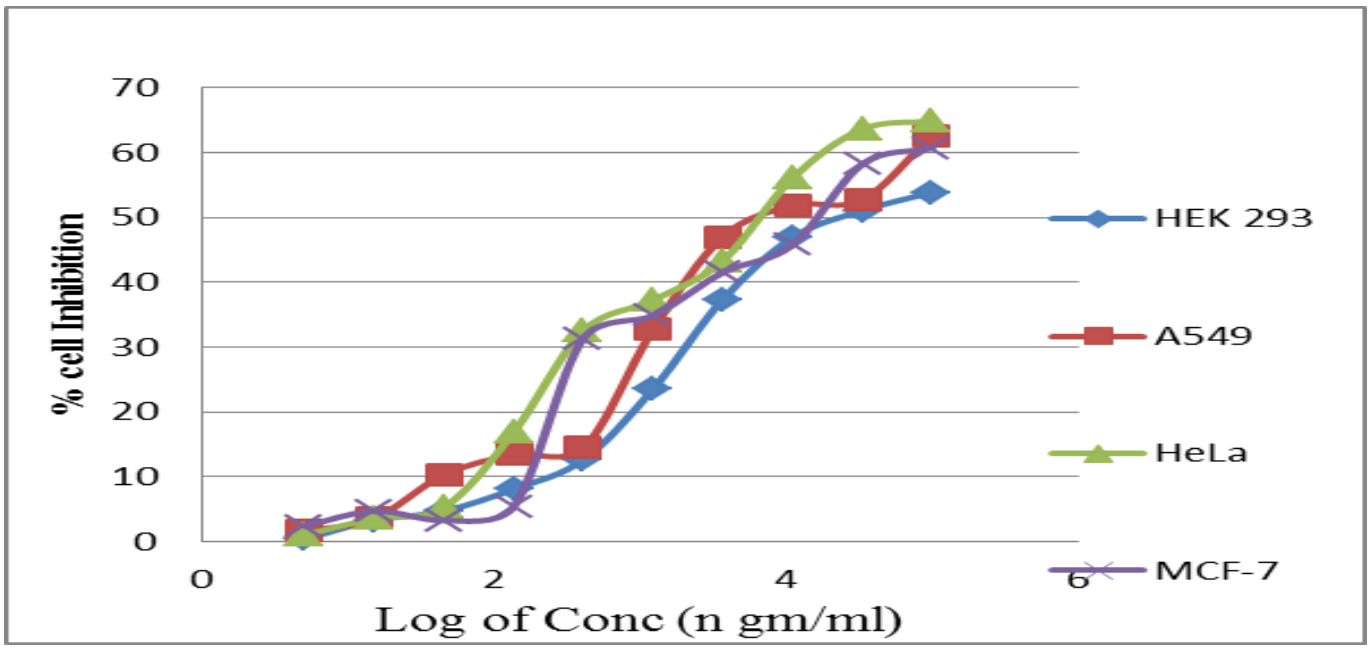


**Figure 2(a) and (b).** % Growth inhibition of methotrexate and acqueous extract of Opuntia ficus-indica against HEK 293, A549, HeLa, MCF-7 by SRB assay

**A**




**B**

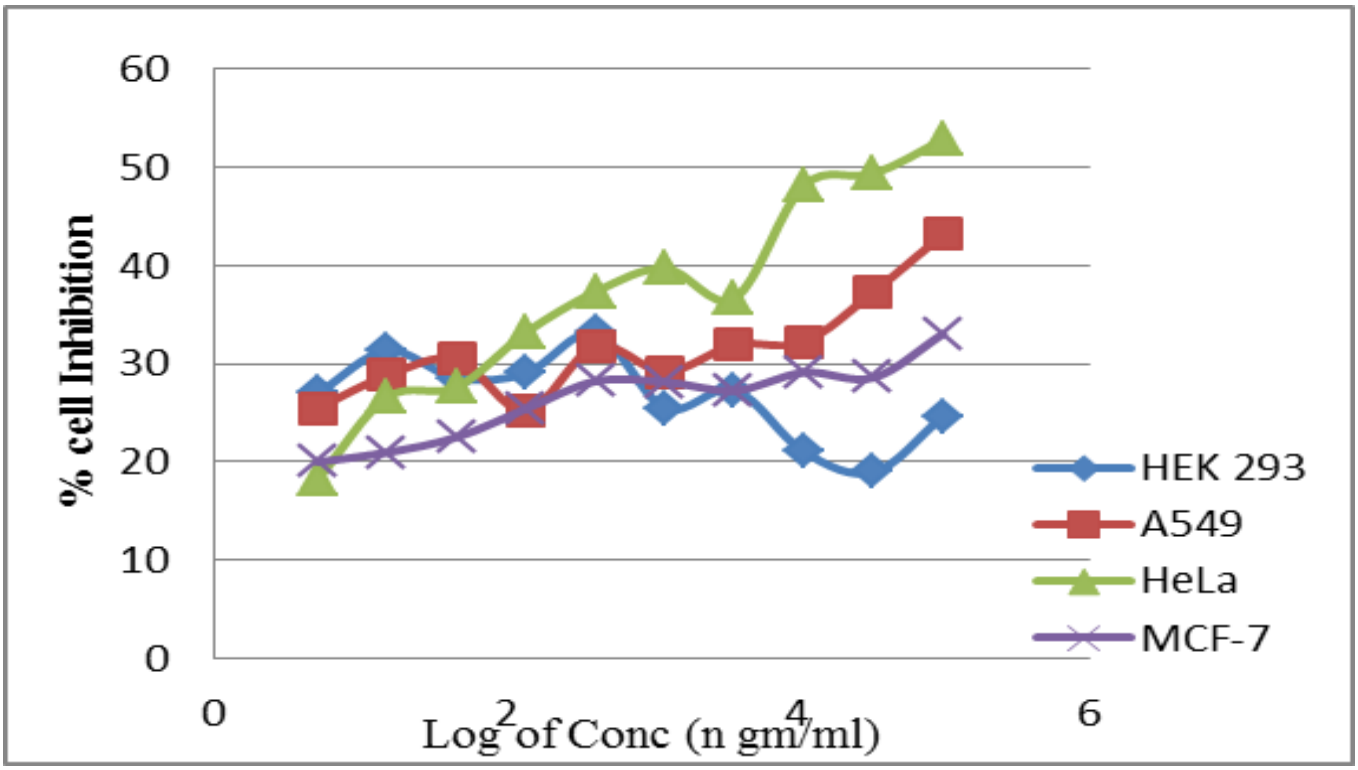


**Table 1. % growth inhibition and $IC_{50}$ values of methotrexate and aqueous extract of Opuntia ficus-indica by MTT assay**

| Compound name | Conc. ng/ml | % Growth Inhibition | | | | IC50 (µg/ml) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | HEK-293 | A549 | HeLa | MCF-7 | HEK-293 | A549 | HeLa | MCF-7 |
| Opuntia ficus-indica | 5.08053 | 26.96915 | 25.485 | 18.21261 | 19.99364 | - | - | 39.72 | - |
| | 15.24158 | 31.3474 | 28.82434 | 26.59811 | 20.95834 | | | | |
| | 45.72474 | 28.52751 | 30.45691 | 27.63702 | 22.5167 | | | | |
| | 137.1742 | 29.04696 | 25.18817 | 33.20259 | 25.41079 | | | | |
| | 411.5226 | 33.12838 | 31.79264 | 37.35821 | 28.23068 | | | | |
| | 1234.568 | 25.485 | 29.19538 | 39.73285 | 28.15647 | | | | |
| | 3703.704 | 27.34019 | 31.94106 | 36.69034 | 27.26598 | | | | |
| | 11111.11 | 21.03255 | 32.23789 | 48.19252 | 29.12117 | | | | |
| | 33333.33 | 18.95473 | 37.35821 | 49.37984 | 28.60172 | | | | |
| | 100000 | 24.66872 | 43.22061 | 52.86759 | 33.05417 | | | | |
| Methotrexate | 5.08053 | 6.8589 | 6.116824 | 16.28326 | 0.996502 | 45.42 | 9.70 | 27.907 | 49.42 |
| | 15.24158 | 18.36107 | 9.530372 | 21.552 | 5.448956 | | | | |
| | 45.72474 | 25.78183 | 14.7249 | 25.41079 | 10.12403 | | | | |
| | 137.1742 | 28.37909 | 26.37549 | 27.63702 | 14.05703 | | | | |
| | 411.5226 | 33.27679 | 36.3193 | 30.30849 | 24.74292 | | | | |
| | 1234.568 | 31.86685 | 42.10749 | 33.72204 | 31.49581 | | | | |
| | 3703.704 | 33.87045 | 46.26312 | 35.13198 | 34.53832 | | | | |
| | 11111.11 | 42.47853 | 50.64137 | 40.99438 | 36.54193 | | | | |
| | 33333.33 | 47.67306 | 55.46486 | 50.64137 | 47.74727 | | | | |
| | 100000 | 56.20693 | 60.43676 | 65.85392 | 59.62048 | | | | |

**Table No. 2. % growth inhibition and $IC_{50}$ values of methotrexate and aqueous extract of Opuntia ficus-indica by SRB assay**

| **Compound name** | **Conc. ng/ml** | **% Growth Inhibition** | | | | **IC50 (µg/ml)** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **HEK-293** | **A549** | **HeLa** | **MCF-7** | **HEK-293** | **A549** | **HeLa** | **MCF-7** |
| Opuntia ficus-indica | 5.08053 | 0.372 | 1.627 | 1.047 | 2.399338 | - | - | 85.63 | - |
| | 15.24158 | 3.268 | 3.557 | 3.557 | 4.71594 | | | | |
| | 45.72474 | 4.715 | 10.3144 | 5.295091 | 3.268064 | | | | |
| | 137.1742 | 8.094 | 13.4032 | 16.8781 | 5.391616 | | | | |
| | 411.5226 | 12.631 | 14.464 | 32.51517 | 31.35687 | | | | |
| | 1234.568 | 23.538 | 32.708 | 37.2449 | 34.83177 | | | | |
| | 3703.704 | 37.341 | 46.993 | 43.32598 | 41.492 | | | | |
| | 11111.11 | 46.897 | 51.820 | 56.06729 | 45.73911 | | | | |
| | 33333.33 | 51.047 | 52.688 | 63.59625 | 58.19084 | | | | |
| | 100000 | 53.847 | 62.631 | 64.85108 | 60.7005 | | | | |
| Methotrexate | 5.08053 | 0.372311 | 1.627137 | 1.047987 | 2.399338 | 45.67 | 16.11 | 7.76 | 16.53 |
| | 15.24158 | 3.268064 | 3.557639 | 3.557639 | 4.71594 | | | | |
| | 45.72474 | 4.71594 | 10.3144 | 5.295091 | 3.268064 | | | | |
| | 137.1742 | 8.094319 | 13.4032 | 16.8781 | 5.391616 | | | | |
| | 411.5226 | 12.631 | 14.46498 | 32.51517 | 31.35687 | | | | |
| | 1234.568 | 23.53833 | 32.70822 | 37.2449 | 34.83177 | | | | |
| | 3703.704 | 37.34142 | 46.99393 | 43.32598 | 41.492 | | | | |
| | 11111.11 | 46.89741 | 51.82019 | 56.06729 | 45.73911 | | | | |
| | 33333.33 | 51.04799 | 52.68891 | 63.59625 | 58.19084 | | | | |
| | 100000 | 53.84721 | 62.631 | 64.85108 | 60.7005 | | | | |

**CONCLUSION**

From the results obtained from MTT and SRB assay methods, by the comparision of the $IC_{50}$ values and linearity of the activity, the methanolic extract of *Opuntia ficus-indica* showed excellent cytotoxicity against the A549 cell line, but had not activity against Hela and MCF-7 cell lines. and HeLa cancerous cell line respectively. The $IC_{50}$ value found for methanolic extract of *Opuntia ficus-indica* on A549 cell line was 35.26 µg/ml and 36.119 µg/ml by MTT and SRB assay respectively. From results it found that the methanolic extract had no cytotoxicity against HEK293, whereas methotrexate showed toxicity with $IC_{50}$ of 45.42 µg/ml and 45.67 µg/ml against HEK 293 by MTT and SRB assay methods respectively. It proved that the methanolic extract of *Opuntia ficus-indica* had potential cytotoxicity against lung cancer, but nontoxic to the normal cells (HEK293 cell line) as compared to methotrexate.

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