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Research Article

# INVITRO CYTOTOXICITY OF VARIOUS PARTS OF *ADHAHAPUSHPI* (*TRICHODESMA INDICUM* LINN.R.BR.) AGAINST 3 CANCER CELL LINES

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## ABSTRACT

Cancer is a leading cause of death and an important source to the mortality and morbidity worldwide. The management in contemporary medicine is having toxicity to normal cells so the world is looking forward for drugs which are cytotoxic to cancer cells and non toxic to host cells and Medicinal plants are doing well in this aspect. *Adhahapushpi (Trichodesma indicum* Linn.R.Br.) Belonging to Boraginaceae family is traditionally used as anticancer to treat breast cancer. Its cytotoxicity against breast cancer and cervical cancer is proved in previous researches. **Objectives:** The present study was conducted to evaluate its cytotoxicity against 3 Human cancer cell lines Viz. colon cancer cell line (HCT116), oral cancer cell line (KB) and skin cancer cell line (A375). **Method:** Alcohol, Methanol and Aqueous extracts of Root, Leaf, Stem and Fruit of *Adhahapushpi* were used to screen in-vitro cytotoxicity. Cytotoxic effect was analysed by MTT assay.
**Results:** The ethanol Root extract was found to be more cytotoxic *in vitro* to colon cancer cell line (HCT116) with IC50values 176.5±13.36µg/ml, ethanol extract of fruit to oral cancer cell line (KB-3-1) with IC50values 154 ± 3.89µg/ml and root ethanol extract to skin cancer cell line (A375) with IC50values 169± 12.09µg/ml respectively.

## INTRODUCTION

Cancer positions as a 1st or 2nd leading cause of death and an important barrier to increasing life expectancy in every country of the world.[1] Worldwide, an estimated 28.4 million new cancer cases (including NMSC, except basal cell carcinoma) are projected to occur in 2040, a 47% increase from the corresponding 19.3 million cases in 2020, assuming that national rates estimated in 2020 remain constant.[2]

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More than 1.9 million new colorectal cancer (including anus) cases and 935,000 deaths were occurred in 2020, it's about one in 10 cancer cases and deaths. Overall, colorectal is third in terms of incidence and second in terms of mortality.[3] About 54,010 new cases of oral cavity or oropharyngeal cancer. About 10,850 deaths from oral cavity or oropharyngeal cancer estimated to occur in 2020[4]. About 115,320 new skin cancer cases and 11,540 deaths were estimated to occur in 2020.[5]

According to WHO, 80% of world's health problems are well treated by herbal drugs only.[6] It is interesting to know that about 25–28% of all modern medicines are directly or indirectly derived from natural plants demonstrating the enormous medicinal potential of plants that has been known for thousands of years in traditional medicine[7].

Modern treatment modalities of cancer like chemotherapy and radiation are said to be with risk of life threatening host toxicity[8]. An ideal anticancer

drug will specifically be cytotoxic only toward the cancer cells and based on the research findings it is proved that the phytochemicals & derivatives present in plants are promising option for the improved and less toxic cancer therapy. Bioactive phytochemicals possess various therapeutic functions (e.g., analgesic, anti-inflammatory, antitumor and many more)[9].

*Trichodesma indicum* Linn.R.Br is rich source of secondary metabolites like Flavanoids, Tannins, Triterpenoids etc. Rutin, Gallic acid and Quercitin like flavones are found in different extracts of plant which acts as cytotoxic against colon, skin and oral cancer.[10]

Acharya Charaka has explained no drug in this world is without medicinal properties, every drug is potential to cure one or the other disorders[11]. In Chattisagarh state, tribal people are using *Adhahapushpi* (*Trichodesma indicum* Linn.R.Br) for the treatment of breast cancer.[12]. *Trichodesma indicum* Linn.R.Br belonging to the family, Boraginaceae[13] is an annual erect, much branched very hispid herb. It is distributed throughout the greater part of India, in the plains, Baluchisthan, Ceylon, Afghanistan, Persia, and Mauritius. Ayurvedic texts explain its properties as: *Laghu Guna, Tikta* and *Katu rasa*, *Ushnavirya*, it is *Kapha Vatahara* and is excellent healer of *Vrana* and *Shotha*.[14] *Adhahapushpi* (*Trichodesma indicum* Linn.R.Br) is been proved for its antioxidant property.

Research works on its anticancer activity has been carried out against human breast cancer, cervical cancer and laryngeal epithelial cell lines.[15] So in the present study, in vitro anticancer activity against colon cancer, oral cancer and skin cancer cell lines will be analyzed.

In this regard, different parts (Root, Leaves, Stem) of *Adhapushpi* (*Trichodesma indicum* Linn.R.Br,) are selected to evaluate its in vitro cytotoxicity against 3 human cancer cell lines viz., colon cancer (HCT116), skin cancer (A375) and oral cancer (KB-3-1).

## MATERIAL AND METHODS

**Collection of plant material:** The plant *T. indicum Linn,* R.Br is collected from Dhanvantari vana of S.G.V.Ayurvedic Medical College Bailhongal and is authenticated by RCMR Belganvi, voucher specimen PHD/SDB/AAMC-11 was deposited to the Department of Dravyaguna vignana, Alvas Ayurvedic Medical College Moodbidri.

### Preparation of Extraction

### Extraction procedure using organic solvents by Successive soxhlet apparatus[16]

The Root, Leaf, Stem and Fruit parts of the plant were washed, air dried for two days. The dried plant's parts were ground into coarse powder. The powdered plant material was subjected separately to successive solvent extraction in Methanol and Ethanol solvents.15gms of powdered plant material was subjected to soxhalet extraction for 8 hrs. with 250ml of the above solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in an air tight container for the analysis of antioxidant activity.

### Extraction procedure using water

For aqueous extraction, 10 g of air-dried powder of different parts of plants was added to distilled water and boiled on slow heat for 2 hours. It was then filtered through cotton cloth and centrifuged at 5000g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours was pooled together and concentrated to make the final volume one-fourth of the original volume 12.

### Cytotoxicity –MTT assay[17]

**Cancer cell line:** The human colon cancer cell line (HTC 1160), Human oral cancer cell line (KB-3-1) and Human skin cancer cell line (A375)was obtained from National Centre for Cell Science, Pune, and grown in Eagles minimum essential medium containing 10% fetal bovine serum (FBS). All the cells were maintained at 37°C, 5% $CO_2$, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Dulbecco`s Modified Eagle Media (DMEM) with low glucose-Cat No-11965-092 (Gibco, Invitrogen) Fetal bovine serum (FBS) - Cat No -10270106 (Gibco, Invitrogen)

Antibiotic – Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062

### Protocol of Cytotoxicity

The cells were seeded in a 96-well flat-bottom micro plate and maintained at 37ºC in 95% humidity and 5% CO2 for overnight. Different concentration (500,250,125,62.5,31.25, 15.62 µg/ml) of samples were treated. The cells were incubated for another 48 hours. The wells were washed twice with PBS and 20 µL of the MTT staining solution was added to each well and plate was incubated at 37ºC. After 4h, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using micro plate reader.

### Formula

Surviving cells (%)= Mean OD of test compound / Mean OD of Negative control ×100, IC 50 of Nonlinear regression graph was plotted between % cell inhibition and $Log_{10}$ concentration and $IC_{50}$ was determined using graph Pad Prism Version5.1software (GraphPad, San Diego, CA).

**Statistical analysis**

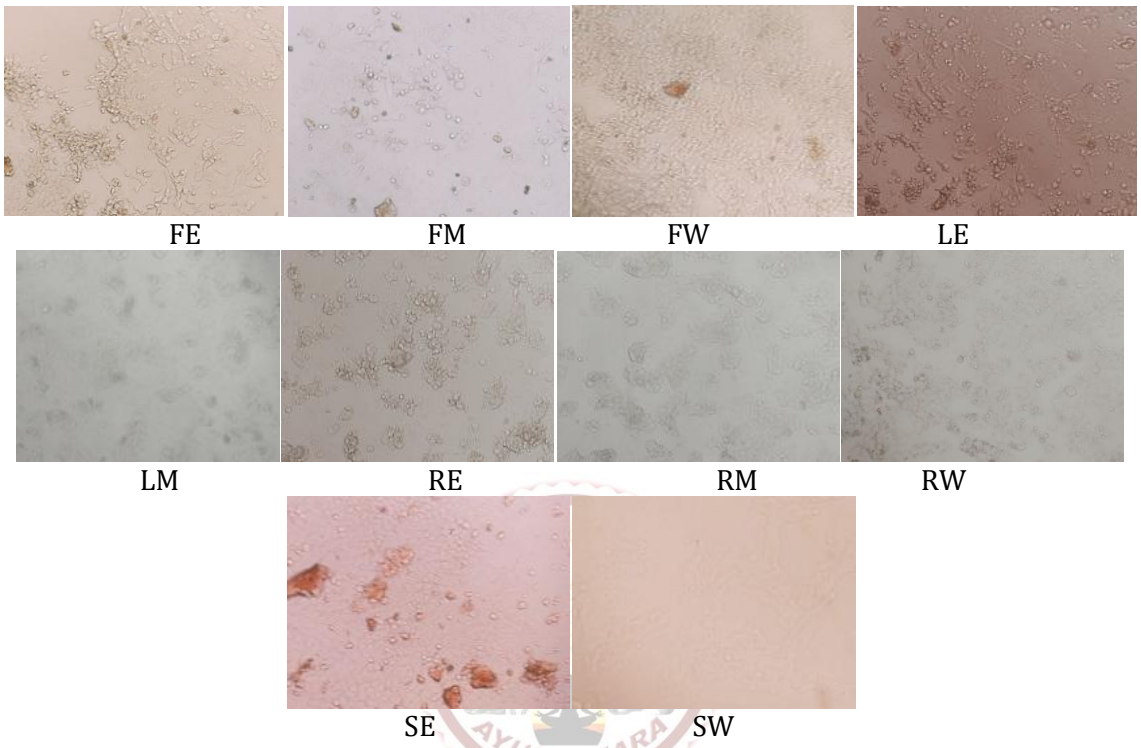
The results were expressed as mean ± standard deviation. Descriptive statistics was used to analyze the mean, standard deviation, variation, and level of statistical significance between groups. When $p < 0.05$ and $p < 0.01$, it was considered statistically significant for analysis of percent cell viability.

**RESULTS AND DISCUSSION**

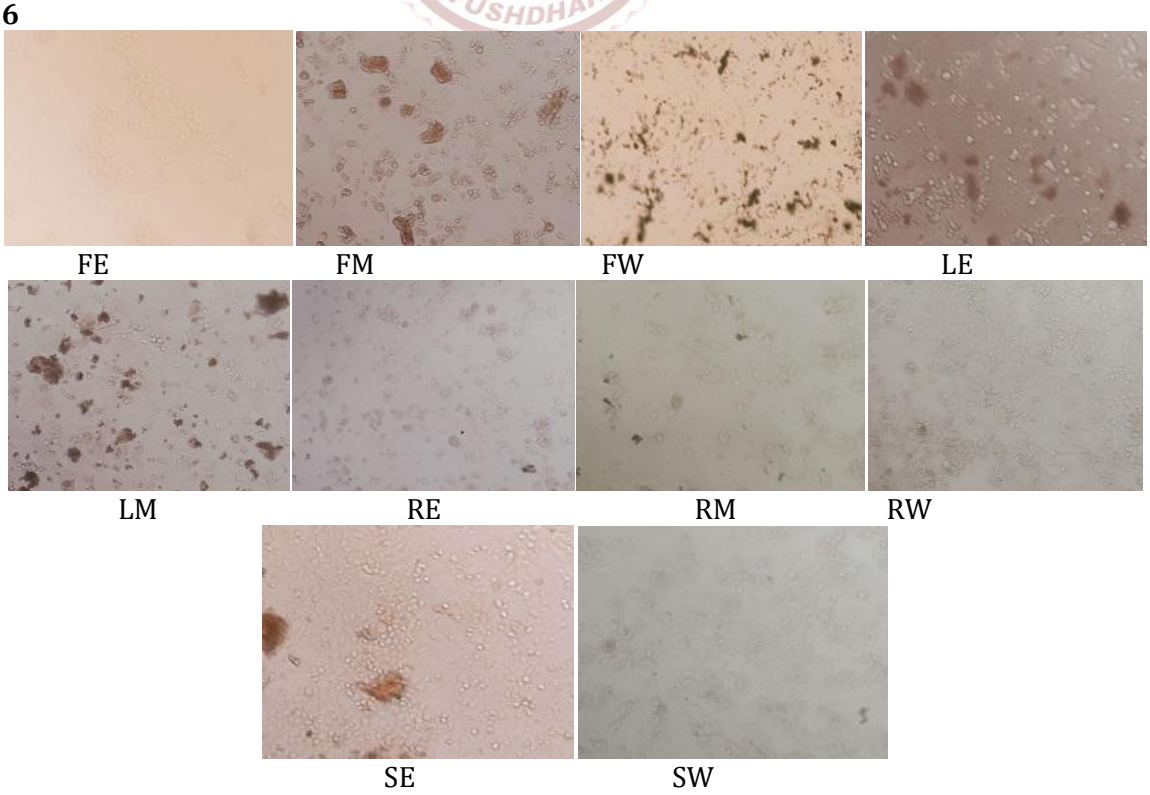
The Results of the study were based on experimental values that were performed in triplicate. Non linear regression graph was plotted between % Cell inhibition and Log10 concentration. The IC50 was determined using Graph Pad Prism software (version 3.00.

**Fig. No. 1 Morphological changes of cell lines after treatment with different extracts**

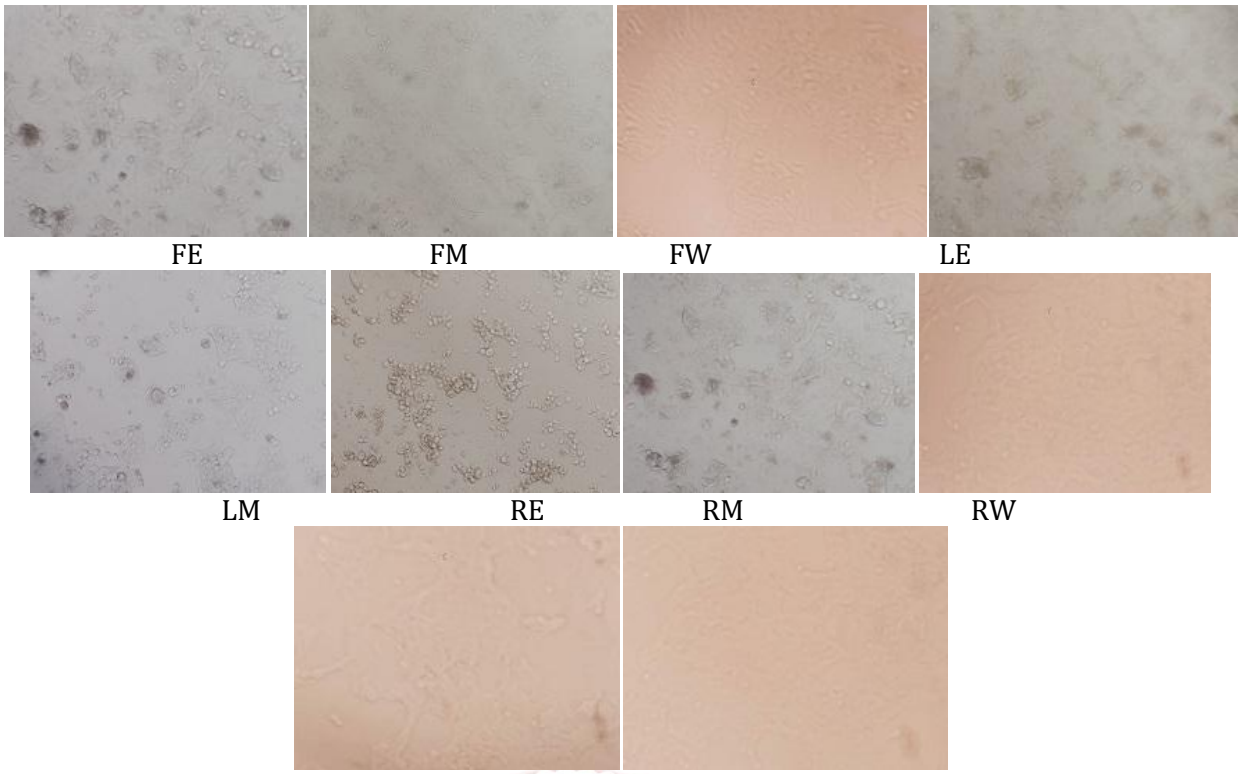
**A. KB-3-1**




**B. HTC 116**



**C. A375**




FE: Fruit ethanol extract, FM: Fruit methanol extract, FW: Fruit Aqueous extract, LE: Leaf ethanol extract, LM: Leaf methanol extract, LW: Leaf aqueous extract, RE: Root ethanol extract, RM: Root methanol extract, RW: Root aqueous extract, SW: Stem aqueous extract, SE: Stem ethanol extract

**Table 1: IC50 values of Different extracts**

| S. No. | Sample Code | KB-3-1 | HTC 116 | A375 | P value |
|---|---|---|---|---|---|
| 1 | FE | 154 ±3.89 | 297.6 ±12.98 | 288.6 ±11.43 | <0.0001* |
| 2 | FM | 256.5 ±8.49 | 192.4 ±0.85 | 182.4 ±1.14 | <0.0001* |
| 3 | FW | 512.9 ±10.25 | 432.3 ±17.09 | 432.1 ±9.68 | <0.0001* |
| 4 | LM | 268.1 ±7.41 | 178.4 ±11.03 | 173 ± 6.01 | <0.0001* |
| 5 | LE | 213.9 ±8.56 | 214.7 ±7.85 | 208.3 ±7.78 | 0.3521 |
| 6 | RE | 285.4 ±6.92 | 176.5 ±13.36 | 169 ±12.09 | <0.0001* |
| 7 | RM | 240.1 ±5.77 | 271.7 ±14.71 | 266.4 ±14.42 | 0.0009* |
| 8 | RW | 318.6 ±5.46 | 799.3 ±13.08 | 656.9 ±13.01 | <0.0001* |
| 9 | SW | 300.9 ±8.87 | 473 ±8.91 | 462.8 ±6.29 | <0.0001* |
| 10 | SE | 332.6 ±6.32 | 235.2 ±10.70 | 226.7 ±10.35 | <0.0001* |

*Statistically Significant, n=3,

FE: Fruit ethanol extract, FM: Fruit methanol extract, FW: Fruit Aqueous extract, LE: Leaf ethanol extract, LM: Leaf methanol extract, LW: Leaf aqueous extract, RE: Root ethanol extract, RM: Root methanol extract, RW: Root aqueous extract, SW: Stem aqueous extract, SE: Stem ethanol extract.

**Fig. No. 1Cell viability of different extracts against 3 cell lines**

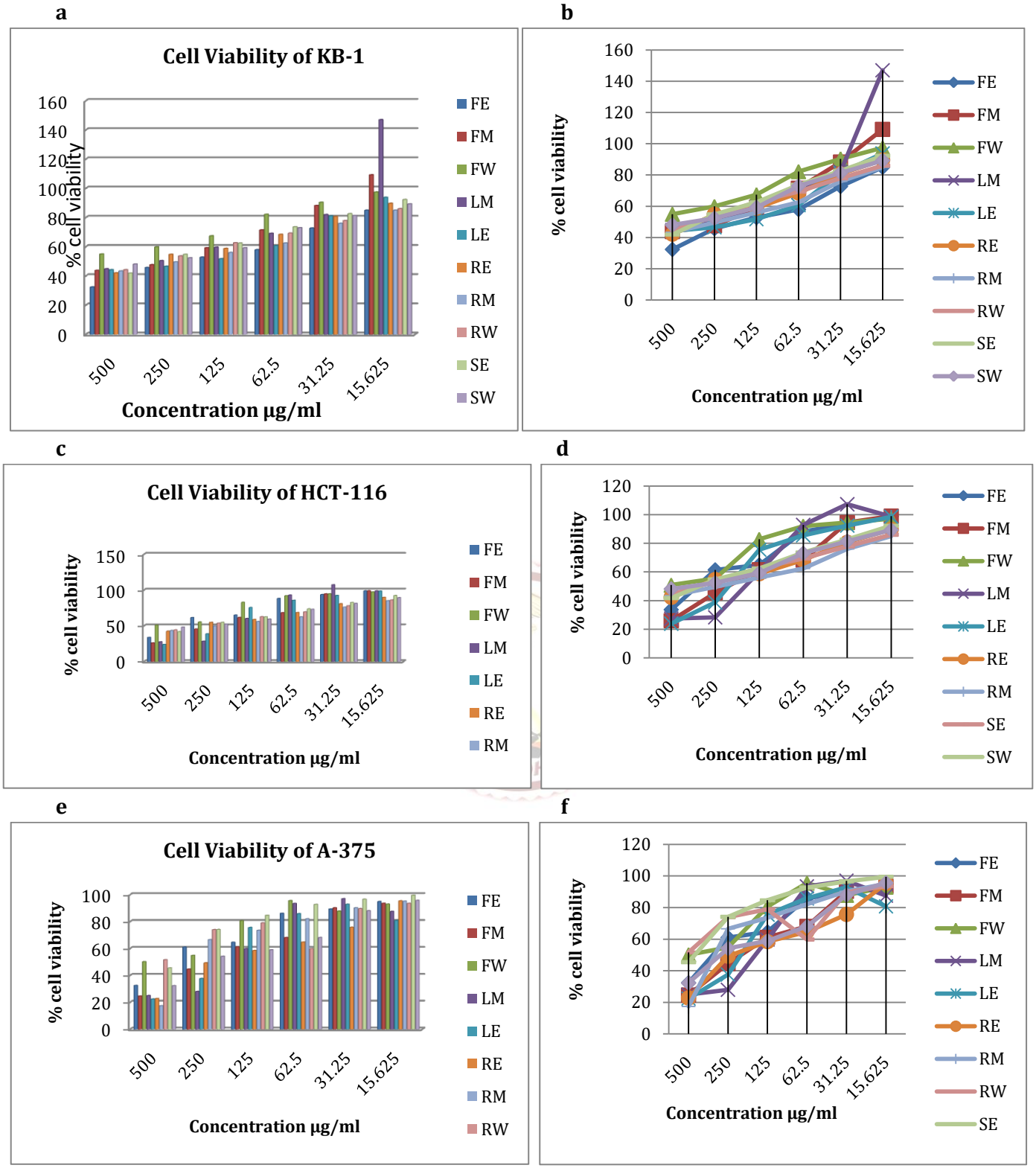



Fig. 1The MTT assay results of different extracts of Adhahapushpi against 3 human cell lines.
a. and b.: cellviability %of KB-3-1. Cand d: cell viability %of HCT-116. e and f: cellviability %of A-375.

Ho: There is no significant relationship between different parts extracts & cell viability.

H1: There is a significant relationship between different parts extracts & cell viability.

**Table 2: ANOVA summery of the effect of different extracts of fruit, leaf, root and stem on the tested cancer cell lines**

| | Cell Viability of KB-3-1 | | Cell Viability of HCT-116 | | Cell Viability of A375 | | ANOVA | P VALUE |
|---|---|---|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | Mean | ±SD | | |
| FE | 86.67 | 28.633 | 73.50 | 24.777 | 71.33 | 23.585 | 0.623 | 0.550 |
| FM | 102.50 | 34.622 | 65.83 | 28.252 | 63.83 | 26.393 | 3.167 | 0.071 |
| FW | 113.33 | 25.967 | 78.83 | 20.615 | 77.00 | 19.647 | 5.077 | 0.021* |
| LM | 112.00 | 54.925 | 69.00 | 35.905 | 37.67 | 13.952 | 5.570 | 0.016* |
| LE | 94.00 | 29.672 | 69.17 | 30.440 | 66.00 | 28.893 | 1.602 | 0.234 |
| RE | 98.00 | 26.367 | 65.83 | 17.612 | 61.00 | 24.470 | 4.539 | 0.029* |
| RM | 92.50 | 23.906 | 62.00 | 15.912 | 70.67 | 28.261 | 2.739 | 0.097 |
| RW | 97.83 | 22.947 | 65.67 | 15.423 | 74.83 | 16.473 | 4.772 | 0.025* |
| SE | 101.00 | 27.734 | 68.00 | 18.536 | 44.00 | 10.844 | 11.981 | 0.001* |
| SW | 100.50 | 24.753 | 67.00 | 16.529 | 66.17 | 23.396 | 4.818 | 0.024* |
| *: Statistically significant | | | | | | | | |

FE: Fruit ethanol extract, FM: Fruit methanol extract, FW: Fruit aqueous extract, LE: Leaf ethanol extract, LM: Leaf methanol extract, LW: Leaf aqueous extract, RE: Root ethanol extract, RM: Root methanol extract, RW: Root aqueous extract, SE: Stem ethanol extract, SW: Stem aqueous extract

## DISCUSSION

The cancer being second cause for death and its the need of the hour to detect cytoxic property of medicinal plants as chemotherapy and other treatment modalities of contemporary medicine are having adverse effects. The plants rich in Biomedicals and with antioxidant property are proved to be anticancer and it is noted from the previous study that the study drug *Adhahapushpi* (*Trichodesma indicum* Linn R.Br.) is rich in phenolics and flavanoids and is proved antioxidant. So in this study its in vitro cytotoxic effect is tested against most common cancers colon, skin and oral cell lines.

MTT Assay is the most commonly used test to assess in vitro anticancer activity. The powder of different parts of plant was extracted using different solvents of increasing polarity viz methanol, ethanol and water. The obtained extracts were studied for *in vitro* cytotoxicity against 3 cell lines. The ethanol Root extract was found to be more *in vitro* cytotoxic to colon cancer cell line (HCT116) with IC50values 176.5±13.36 µg/ml, ethanol extract of fruit to oral cancer cell line (KB-3-1) with IC50values 154 ± 3.89 µg/ml and root ethanol extract to skin cancer cell line (A375) with IC50values 169± 12.09 µg/ml respectively.

Further statistical analysis by ANOVA at $P < 0.05$ was set to be the limit of significance. It is inferred that there is a significant influence of the treated extracts of different parts of *Adhahapushpi* on the cyto viability of the tested human cancer cell lines, so H1 is accepted and H0 is rejected.

## CONCLUSION

The data obtained in the study suggest that the extracts obtained from the different parts of selected plant *Adhahapushpi (Trichodesma indicum* Linn R.Br.) has anticancer property so *Adhahapushpi (Trichodesma indicum* Linn R.Br.) could be considered as a significant source of natural anticancer.

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