Prakash. J et al /J. Pharm. Sci. & Res. Vol. 11(6), 2019, 2353-2356

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# *In-vitro* Cytotoxicity Studies of Methanolic Bulb Extract of *Zephyranthes Citrina* on Cervical Cancer (Hela), Breast Cancer (MCF-7) and Oral Cancer (SCC-9)

**Prakash. J[1] and Vedanayaki. S[1]***

[1,1*]*Dept. of Chemistry, Kandaswami Kandar's College, Velur, Namakkal – 638 182.*

**Abstract**

Medicinal plants play a vital role to cure many human diseases from thousands of years ago. Cancer is one of the most killing diseases and causes cruel defects on human being. There are many types of cancer diseases which affects the different organs of the body. Yet there is no appropriate medicine to cure such type of cancer diseases. In the present research work methanolic bulb extract of *Zephyranthes citrina* is tested for *in-vitro* cytotoxic activity against three different human cancer cell lines such as cervical cancer (HeLa), breast cancer (MCF-7) and oral squamous carcinoma cancer (SCC-9). The cytotoxicity studies of the extract on cancer cell lines were investigated *in-vitro* through MTT assay. The results revealed that increase in the concentration of the extract, the percentage of inhibition become increases. The changes in the cell lines after exposure with the extract were observed under phase contrast microscope at various dose dependent manners. Among the three cell lines tested, methanolic bulb extract of *Zephyranthes citrina* showed significant cytotoxic effects on SCC-9 cell lines having an $IC_{50}$ value of 88.79 µg/ml.

**Keywords:** Cytotoxicity, *Zephyranthes citrina*, HeLa, MCF-7, SCC-9, MTT Assay

## INTRODUCTION

Phytochemistry is the branch of chemistry concerned with plants and plant products. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years[1]. Medicinal plants are most significant source of life saving drugs for a majority of the world's population[2]. The world Health Organization (WHO) estimates that 80% of the populations of developing counties rely on traditional medicines, mostly plant drugs[3]. The valuable medicinal properties of different plants are due to the presence of several chemical constituents like alkaloids, tannins, phenolics, flavonoids, terpenoids, carbohydrates, glycosides, steroids, saponins, fats and oils etc., among them, some are synergistic and enhance the bioactivity of other compounds[4].

*Zephyranthes citrina* was described by Baker in 1882. It belongs to the family Amaryllidaceae. Plants of the Amaryllidaceae family, a small group of monocotyledonous species, include about 860 to 1100 species in eighty five genera distributed largely over tropical and sub- tropical regions[5]. It is a bulbous plant with green leaves dull 4mm wide. The one-inch lemon yellow flowers of this rain lily spring forth in late summer. These yellow blooms face upwards and fare open, giving them a cheerful appearance[6]. It grows luxuriantly in natural grasslands and as well as in gardens after rain fall. Since they often come into bloom after it rains, *zephyranthes* are commonly called as citron *zephyr lily* or *yellow rain lily*[7]. The main objective of the present study is to evaluate cytotoxicity of bulb methanolic extract of *zephyranthes citrina* on three different human cancer cell lines such as cervical cancer (HeLa), breast cancer (MCF-7) and oral squamous carcinoma cancer (SCC-9).

Natural products and connected drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorder[8]. More than 3000 plant species have been reported to have anticancer properties[9]. India has a rich heritage of traditional knowledge and is home to several important time – honored systems of health care like Ayurveda, Siddha and Unani.

Cancer is a leading cause of death worldwide, accounting for an estimated 9.6 million deaths in 2018. The most common cancers are lung, breast, cervical, liver, prostate, oral and colorectal cancer[10]. Cancer is a wide group of diseases involving abnormal cell growth with the potential to invade nearby parts of the body. It is one of the most prominent diseases in humans and is still not curable in many cases[11]. HeLa (Epithelial Cervical cancer) is an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line. The cell line was derived from cervical cancer cells on 1951[12]. MCF-7 (Breast cancer) is the second cancer effect of people worldwide and the most common cancer among femals[13]. It is isolated in 1950 from a 69-year old woman. MCF-7 is the acronym of Michigan Cancer Fondation-7. SCC-9 (Oral cancer) is the sixth most common type of cancer in the world. Squamous cell carcinomas (SCC) make up 96% of all oral cancer. The poor prognosis of SCC is owing to invasion and local reappearance. SCC cells undergo a transformation process known as the epithelial – to – mesenchymal transition[14].

## MATERIALS AND METHODS

### Collection and Authentication of Plant material

The plant was collected from host area of Institution and Kuchipalayam, Paramathi velur, Namakkal District, Tamil Nadu, India.. The plant was identified and authenticated in the BSI (Botanical Survey of India), Department of Botany, Agricultural University, Coimbatore. The bulbous plant material were cut into pieces, dried under shade for 15 days, coarsely powdered and stored in air tight containers for the further study.

**Preparation of plant extract by Soxhlet extraction**

The *Zephyranthes citrina* sample was dried and successfully extracted with methanol in a Soxhlet apparatus by Soxhlet extraction method. The concentrated extract was subjected to preliminary qualitative analysis for the identification of various phytochemical constituents as per standard procedures[15-17].

**Preparation of test solutions**

For cytotoxicity studies, 10 mg of the sample (*Zephyranthes citrina* methanolic bulb extract) was dissolved in 100 µl of DMSO (Dimethyl sulphoxide) to obtain a main stock solution of concentration 100 mg/ml. From this serial dilutions (25, 50, 100, 200, 400, 600, 800 and 1000 µg / ml) were prepared for cytotoxicity studies.

**Cell lines and culture medium**

The cell lines [HeLa (Cervical cancer), MCF-7 (Breast cancer) and SCC-9 (Oral cancer)] were procured from American Type Culture Collection (ATCC), Manassas, Virginia. The stock cells were cultured in Dulbecco's Modified eagle Medium (DMEM) / Nutrient Mixture F-12 (F 12) supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin 100 IU/ml, streptomycin 100 µg/ml in a humidified atmosphere of 5 % $CO_2$ at 37˚C. The cells were dissociated with Trypsin Phosphate Versene Glucose (TPVG) dissociating solution [0.2 % trypsin, 0.02 % EDTA and 0.05 % glucose in Phosphate Buffer Saline (PBS)]. The stock cultures were grown in 25 $cm^2$ culture flasks and all experiments were carried out in 96 microtitre plates[18-23].

**Cytotoxicity evaluation by MTT assay**

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x $10^5$ cells / ml using DMEM medium containing 10 % FBS. 100 µl of the diluted cell suspension (approximately 10000 cells / well) was added, to each well of the 96 well microtitre plates. The supernatant was flicked off, the formed monolayer washed with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. Then, the plates were incubated at 37˚C for 24 hrs in 5 % $CO_2$ atmosphere. After incubation, the test solutions in the wells were discarded and 100 µl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (5 mg/10 ml of MTT in PBS) was added to each cell. The plates were incubated for 4 hrs at 37˚C in 5 % $CO_2$ atmosphere. The supernatant was removed, 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance (OD) was measured at a wavelength of 590 nm. The percentage of growth inhibition was calculated using the formula given below[18-23].

**% Inhibition = 100 – [Absorbance (OD) of the sample / Absorbance (OD) of the control] X 100**

**Statistical evaluation ($IC_{50}$ value)**

The half maximal inhibitory concentration ($IC_{50}$) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. The required concentration for a 50 % of inhibition was determined from the dose response curves of each cell line. $IC_{50}$ values for cytotoxicity tests were derived from a nonlinear regression analysis.

## RESULTS

The cytotoxicity results of the bulb methanolic extract of *Zephyranthes citrina* on three different cell lines are expressed in the table as given below.

**Cell line: HeLa (Cervical Cancer) –** Table 1: Cytotoxicity of *Z.citrina* on HeLa cell line

| Compound name | Conc. µg/ml | OD at 590nm | % of Inhibition | $IC_{50}$ µg/ml |
|---|---|---|---|---|
| Control | 0 | 0.7651 | 0 | |
| | 25 | 0.6278 | 17.94 | |
| | 50 | 0.5167 | 32.47 | |
| | 100 | 0.4314 | 43.62 | |
| ***Z.citrina ME*** | 200 | 0.3047 | 60.17 | **136.4** |
| | 400 | 0.1927 | 74.82 | |
| | 600 | 0.1457 | 80.96 | |
| | 800 | 0.1116 | 85.42 | |
| | 1000 | 0.0946 | 87.63 | |

**Cell line: MCF-7 (Breast Cancer) –** Table 2: Cytotoxicity of *Z.citrina* on MCF-7cell line

| Compound name | Conc. µg/ml | OD at 590nm | % of Inhibition | $IC_{50}$ µg/ml |
|---|---|---|---|---|
| Control | 0 | 0.8232 | 0 | |
| | 25 | 0.6591 | 19.94 | |
| | 50 | 0.5366 | 34.81 | |
| | 100 | 0.4131 | 49.82 | |
| ***Z.citrina ME*** | 200 | 0.3097 | 62.38 | **104.8** |
| | 400 | 0.2207 | 73.19 | |
| | 600 | 0.1687 | 79.51 | |
| | 800 | 0.1246 | 84.86 | |
| | 1000 | 0.0964 | 88.29 | |

**Cell line: SCC-9 (Oral Cancer) –**T able 3: Cytotoxicity of *Z.citrina* on SCC-9 cell line

| Compound name | Conc. µg/ml | OD at 590nm | % of Inhibition | $IC_{50}$ µg/ml |
|---|---|---|---|---|
| Control | 0 | 0.7846 | 0 | |
| | 25 | 0.6033 | 23.11 | |
| | 50 | 0.4737 | 39.63 | |
| | 100 | 0.3553 | 54.71 | |
| ***Z.citrina ME*** | 200 | 0.2368 | 69.82 | **88.79** |
| | 400 | 0.1549 | 80.26 | |
| | 600 | 0.0912 | 88.37 | |
| | 800 | 0.0665 | 91.52 | |
| | 1000 | 0.0562 | 92.84 | |

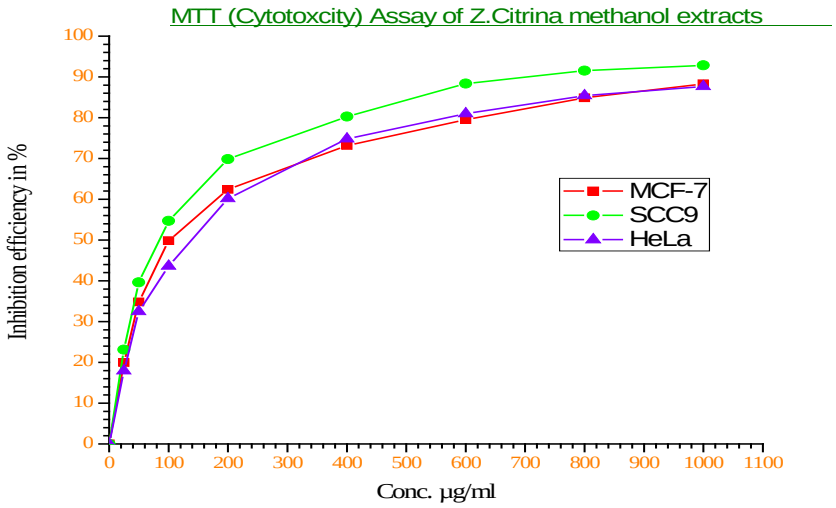


**Figure 1:** The % of inhibition against three cell lines at various concentrations (25 – 1000 µg/ml) with control

Figure 2a:Cytotoxic Activities of *Z.citrina* against HeLa cell lines at two concentrations with control

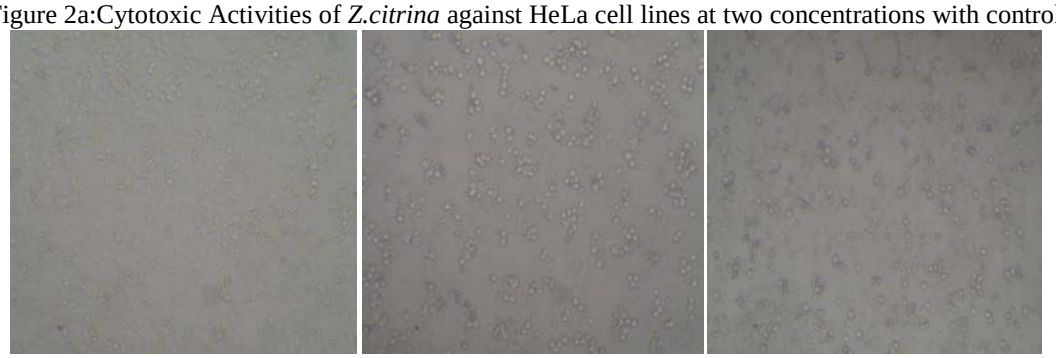


Figure 2b: Cytotoxic Activities of *Z.citrina* against MCF-7 cell lines at two concentrations with control

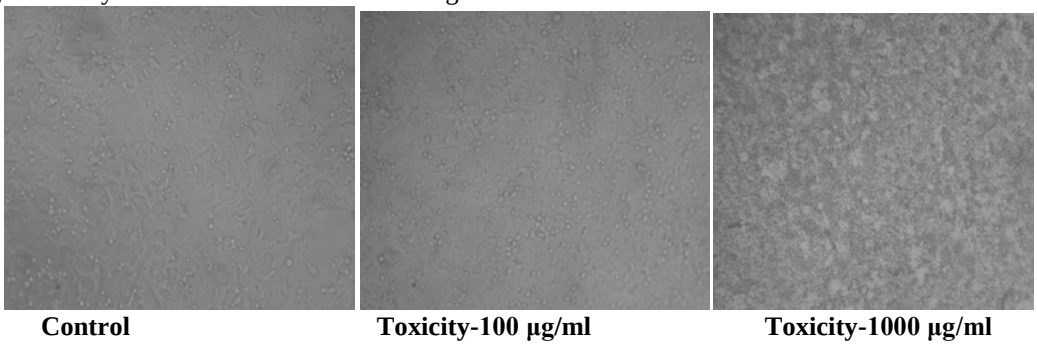


Figure 2c: Cytotoxic Activities of *Z.citrina* against SCC-9 cell lines at two concentrations with control

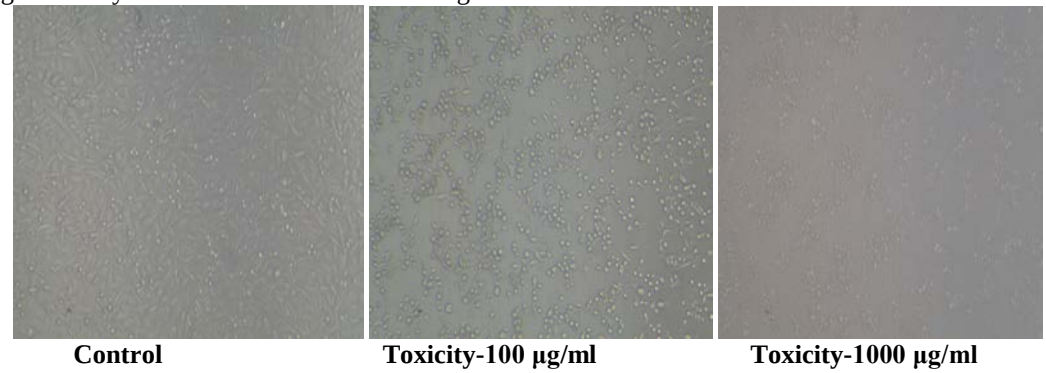


**Figure 2: Cytotoxic Activities of *Z.citrina* against three cell lines at two concentrations with control**

## DISCUSSION

The percentage of inhibition against three different human cell lines (HeLa, MCF-7 and SCC-9) with crude methanolic bulb extract of *Zephyranthes citrina* (Compound Name) showed variations in activity. In all the cell lines, the concentrations of the sample like 25, 50, 100, 200, 400, 600, 800 and 1000 µg/ml of the sample are tested. The absorbance (OD) is measured using a microplate reader at a wavelength of 590 nm. Different concentrations of the sample exhibited different activity on different cell lines. The results revealed that the % of inhibition of the cell lines increases with increase in concentration of the sample in µg/ml.

The % of inhibition (cytotoxic activity) against HeLa, MCF-7 and SCC-9 human cancer cell lines is observed and the results obtained are tabulated in table 1, table 2 and table 3 respectively. The graphical representation of the % of inhibition efficiency against concentrations in µg/ml is expressed in figure 1. After exposure of extract to each cell lines, the percentage of cell viability are observed under phase contrast microscope at two different concentrations such as 100 and 1000 µg/ml with control. The results are shown in figure 2 which include figure 2a, 2b and 2c.

The concentration of the sample required to inhibit cell growth by 50 % called $IC_{50}$. The $IC_{50}$ values of a sample are determined by constructing a prism dose-response curve for every cell lines. Among the three cell lines tested, SCC-9 showed higher activity ($IC_{50}$ – 88.79 µg/ml) than the others. The other two cell lines, MCF-7 ($IC_{50}$ – 104.8 µg/ml) and HeLa ($IC_{50}$ – 136.4 µg/ml) also found to be possess significant cytotoxic activity.

## CONCLUSION

Based on the results obtained in the present investigation of *in-vitro* cytotoxicity studies of the crude methanolic bulb extract of *Zephyranthes citrina* against three different human cancer cell lines (HeLa, MCF-7 and SCC-9), it conifers the presence of cytotoxic compound or tumor degrading substances. The results revealed that SCC-9 cell line showed a potent cytotoxicity with $IC_{50}$ value of 88.79 µg/ml. In accordance with the findings from the report, the phytochemical constituents such as flavonoids, alkaloids, phenolics and terpenoids are the major components which are responsible for the potential cytotoxic activity. Further research is to be carried out to fractionate, inorder to find out the molecules responsible for cytotoxic activity observed.

## REFERENCES


1. Babu. K, Samundeeswari. A and Chitti Babu. C.V. *Int. J. Curr. Sci.*, 2015, 15 (S), 37-42.
2. Debnath. M, Malik. C.P, Bisen. P.S. Micrpropagation:. *Curr. Pharmaceut. Biotechnol*. 2006, 7, 33-49.
3. Prabakaran. M, Chandrakala. N and Panneer Selvam. A. *Asian J. Plant Sci. Res.*, 2011, 1 (2), 18-25.
4. Yvette. F, Kiyinlma. C and Dieneba. K. *Journal of Pharmacognosy and Phytochemistry*, 2014, 2(5), 74-79.
5. Jin. Z. *Natural Product Reports*, 2016, 33(11), 1318–1343.
6. "Zephyranthes citrina". Natural Resources Conservation Service *PLANTS Database*. USDA. Retrieved 9 August 2015.
7. Wikipedia contributors. "*zephyranthes*" Wikipedia. The free Encyclopedia, 2015.
8. Newman. D.J and Cragg. G.M. *Journal of Natural Products*. 2007, 70 (3), 461-477.
9. Graham. J.G, Quinn. M.L, Fabricant. D.S and Farnsworth. N.R. *Journal of Ethnopharmacology*. 2000, 73 (3), 347-377.
10. Cancer. World Health Organization (WHO). 12 September 2018. Retrieved 19 December 2018.
11. Stewart. BW and Wild. CP. World cancer report, Lyon, *International agency for Research on Cancer*. 2014.
12. Rahbari. R, Sheahan. T, Modes. V, Collier. P. Macfarlane. C and Badge. RM. *Bio Techniques*, 2009, 46 (4), 277-284.
13. Parkin DM, Fernandez LM. Use of statistics to assess the global burden of breast cancer. *Breast J*, 2006, 12 suppl 1: S 70-80.
14. Carlin Lee, Casey Lee, Amha Atakilit, Amanda Siu and Daniel M. Ramos. *International Institute of Anticancer Research*, 2014, 34 (12), 6945-6949.
15. Sofowara. A. Medicinal plants and Traditional medicine in Africa. Nigeria, *Spectrum Books Ltd*, 1993.
16. Trease. GE and Evans. WC. Pharmacognosy, Edition 11, London, *Bailliere Tindall Ltd.*, 1989.
17. Harborne. JB. Phytochemical Methods. London, *Chapman and Hall Ltd.*, 1973.
18. Gonzalez, R.J. and Tarloff, J.B. *Toxicology in vitro*. 2001, 15, 259-265.
19. Pater. MM and Pater. A. Human papillomavirus types 16 and 18 sequences in carcinoma cell lines of the cervix. *Virol*, 1985, 145, 313-318.
20. Matsukawa. Y, Marui. N, Sakai. T, Satomi. Y and Aoike. A. *Cancer Research*, 1993, 53, 1328-1331.
21. Francis. D and Rita. L. *Journal of Immunological Methods*. 1986, 89, 271-277.
22. Kalpana. B and prakash. M. *Inter. J. Curr. Microbio. And App. Sci.* 2017, 6 (8), 3367-3373.
23. Mosmann. T.T. *Journal of Immunological Methods*, 1983, 12-16.