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# IN VITRO CYTOTOXICITY STUDIES ON ISOLATED AND PURIFIED COMPOUNDS FROM CHRYSOPOGON ZIZANIOIDES CHLOROFORM EXTRACT ON VERO AND HCT-116 CANCER CELL LINES

**Sajusha Duguluri*** Research scholar, Department of Bio-Technology, Bharath Institute of Higher Education and Research. *Corresponding Author

**Selvakumar S** Professor, Department of Agricultural Bio-Technology, Bharath Institute of Higher Education and Research.

**ABSTRACT** The main objective of this study is to investigate the cytotoxic potential of four purified compounds (compounds 1,2,3 and 4) from medicinal plant Chrysopogon zizanioides L were investigated. Successive solvent extraction of Chrysopogon zizanioides in chloroform was done. The extract were tested against the normal cell lines (Vero) human colorectal cancer cell lines (HCT-116) using the thiazolyl blue test (MTT) assay. The chloroform fractions of Chrysopogon zizanioides had shown the significant results against Vero and HCT-116 tumor cell lines. The IC50concentration of the selected purified compounds C1, C2, C3, and C4 against HCT 116 were 79.06 µg/ml, 84.13 µg/ml, 94.36 µg/ml and 68.47µg/ml, respectively. Whereas the IC50concentration of the selected purified compounds C1, C2, C3, and C4 against Vero were 130.30µg/ml, 157.36µg/ml, 160.48µg/ml and 255.28µg/ml, respectively. Among all the tested compounds, compound four can be considered as potential sources of anti-cancer compounds. Additional studies are necessary for more extensive biological evaluations.

**KEYWORDS :** Cytotoxicity studies, Vero cells, HCT-116 cells, MTT assay.

## INTRODUCTION

The plant kingdom contains a large number of new biologically active compounds. Due to their inherent biological properties, they can be used in medicine and other fields that promote human health. In general, medical practice, botanical drugs play an important role in the treatment of cardiovascular system diseases [1], nervous system [2] and immune system [3]. In addition, many herbs also have preventive effects. They are used in the treatment of diseases [4]. It is estimated that the development of new anti-cancer drugs will take 7 to 10 years and cost more than 5 million US dollars. This includes the initial collection of samples, the evaluation of crude extracts, the purification, identification and synthesis of active substances, and the costs of preclinical and clinical studies. Many commercially sold medicinal plants may contain chemical substances with potential mutagenic and/or carcinogenic properties [5] and chemical substances with anti-tumor properties [6].

The active extracts detected by screening methods should Perform accurate bioassays to determine its specific pharmacological activity. The increased interest in apoptosis or programmed cell death (PCD) has had a significant impact on many areas of biological sciences, including oncology [7]. The description of discrete apoptotic pathways not only affects our basic concept of cancer development, but also affects our methods of preventing and treating the disease. It is now clear that the balance between cell proliferation and death plays a vital role in maintaining the homeostasis of normal tissues, and any disorder in any of these processes may lead to clonal expansion disorders, which is a characteristic of all neoplastic diseases. [8].

Apoptosis is the main mechanism of action of many standard cytotoxic agents, and this realization has led to new experimental methods aimed at stimulating the apoptotic pathway to improve the response to treatment. It is known that natural compounds have played an important role in regulating cell proliferation and differentiation for more than 50 years, but only recently have they realized their importance as physiological and pharmacological regulators of cell death [9]. Natural products are considered to be important mediators of key pathways in cancer development and progression, and people have renewed interest in their potential as chemoprevention and chemotherapy anticancer drugs [10].

## MATERIAL AND METHODS

### Sample Collection

The medicinal plant used in the experiment is an aerial part of *Chrysopogon zizanioides L* were collected from a local medicinal farm. The plant materials were identified by reputed botanist from Madras Christian College, Tambaram, Chennai, India.

### Preparation Of Extract

1,000 grams of plant material were placed in three separate round-bottom flasks to extract samples using solvents (chloroform). Extraction was performed with 250 ml of solvent mixture for 24 hours. At the end of the extraction, the respective solvent were concentrated under reduced pressure and placed in a water bath (50°C). Now store the extracted experimental solution in the refrigerator.

### Chemicals And Reagents

All chemicals used in this project were purchased from Sigma Chemical Company. USA.

### CYTOTOXICITY Analysis

### Preparation Of Cell Suspension

A subculture of HCT116 and Vero cell lines were in Dulbecco's Modified Eagle's Medium (DMEM) was trypsinized separately, after discarding the culture medium. To the disaggregated cells in the flask 25 mL of DMEM with 10% FCS was added. The Cells suspended in the medium by gentle passage with the pipette and the cells homogenized.

### Seeding Of Cells

One mL of the homogenized cell suspension was added to each well of a 24 well culture plate along with different concentration of tested samples (0- 200 µg/mL) and incubated at 37°C in a humidified $CO_2$ incubator with 5% $CO_2$. After 48 hrs incubation the cells were observed under an inverted tissue culture microscope. With 80% confluence of cells cytotoxicity assay was carried out [11].

### CYTOTOXICITY Assay

The assay was carried out using 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial Succinate dehydrogenase and reductase of viable cells, yielding a measurable purple product of formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. After 48 h incubation the wells were added with MTT (5 µg/ml) and left for 3 h in room temperature. All wells were removed the content using pipette and 100µl DMSO were added to dissolve the formazan crystals, absorbance's were read in Readwel Touch micro plate reader at 570 nm.

## RESULTS AND DISCUSSION

The *in-vitro* cytotoxicity activity studies of pure compounds were tested against HCT 116 and Vero cell lines. Experimental studies have shown that a variety of chemo preventive plant components can affect the occurrence, promotion and development of tumors. The main difference between botanicals and synthetic drugs is the presence of complex metabolite mixtures displayed by botanicals, and the latter has an effect on botanicals played a roleat different levels and through different mechanisms. In this study, we conducted in vitro screening of four purified compounds namely compound 1,2,3 and compound 4 from chloroform extract of *Chrysopogon zizanioides* Experimental results show that the purified compounds especially compound 4 can significantly reduce the proliferation of tumor cells in vitro, indicating that these extracts may be chemical adjuvants for different cancer treatments. Based on the antioxidant or free radical scavenging

potential and in vitro cytotoxic effects of purified compounds from chloroform extract of *Chrysopogon zizanioides L* the compound 4 has highly significant than the other three compounds.

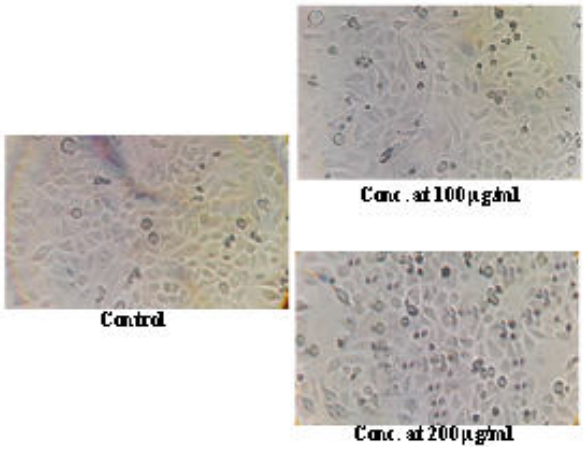


**Figure1:** Cytotoxic effect of purified Compound C1 against HCT 116 cell line

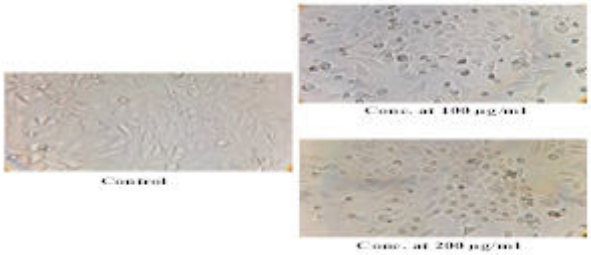


**Figure 2:** Cytotoxic effect of purified Compound C2 against HCT 116 cell line

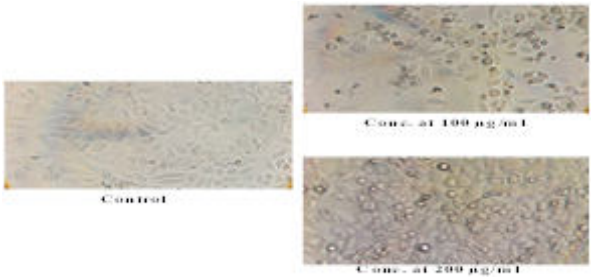


**Figure 3:** Cytotoxic effect of purified Compound C3 against HCT 116 cell line

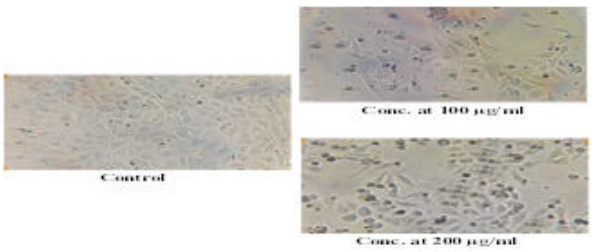


**Figure 4:** Cytotoxic effect of purified Compound C4 against HCT 116 cell line

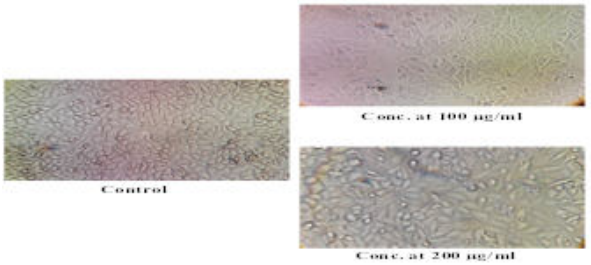


**Figure 5:** Cytotoxic effect of purified Compound C1 against Vero cell line

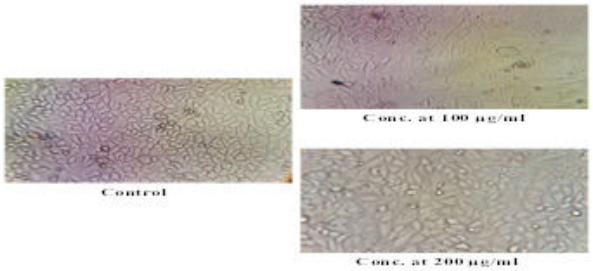


**Figure 6:** Cytotoxic effect of purified Compound C2 against Vero cell line

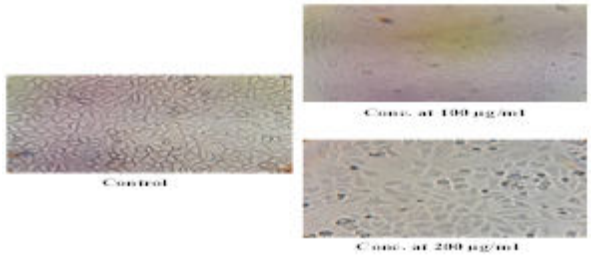


**Figure 7:** Cytotoxic effect of purified Compound C3 against Vero cell line

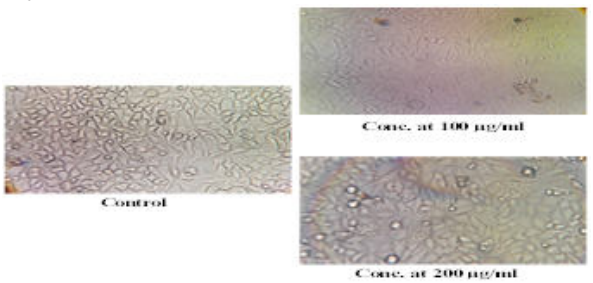


**Figure 8:** Cytotoxic effect of purified Compound C4 against Vero cell line

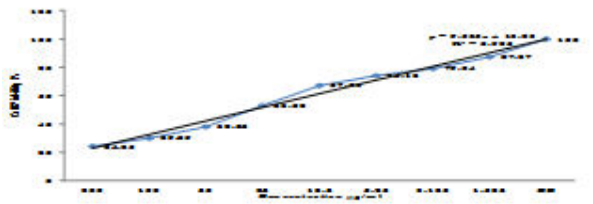

**Figure 9:** Cytotoxic effect of purified compound C1 against HCT 116 cell line

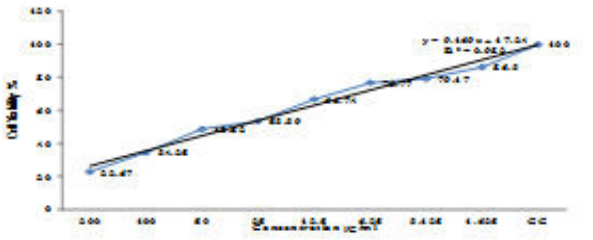

**Figure 10:** Cytotoxic effect of purified compound C2 against HCT 116 cell line

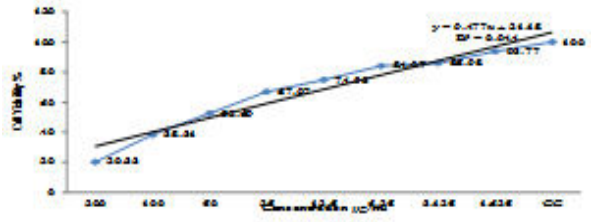

**Figure 11:** Cytotoxic effect of purified compound C3 against HCT 116 cell line

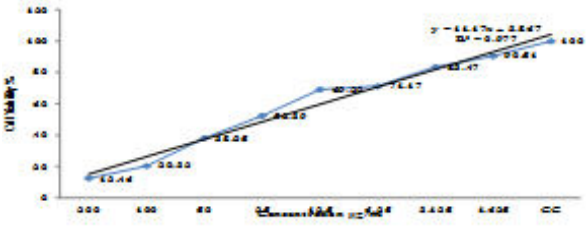

**Figure 12:** Cytotoxic effect of purified compound C4 against HCT 116 cell line

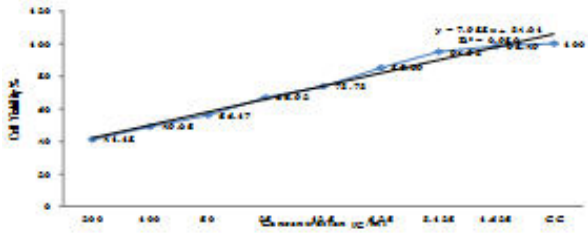

**Figure 13:** Cytotoxic effect of purified compound C1 against Vero cell line

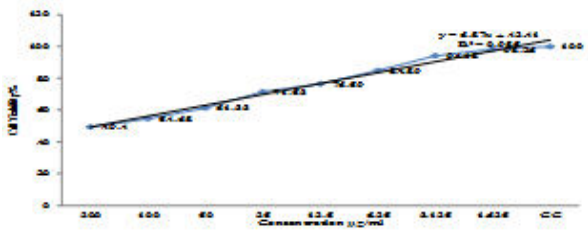

**Figure 14:** Cytotoxic effect of purified compound C2 against Vero cell line

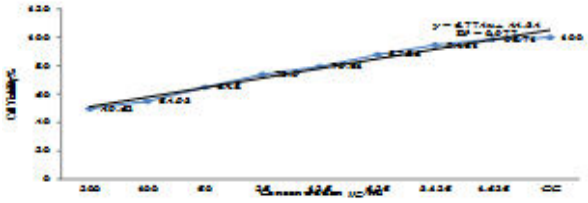

**Figure 15:** Cytotoxic effect of purified compound C3 against Vero cell line

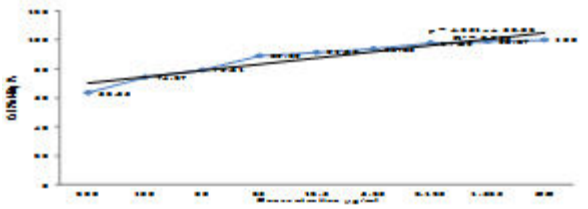

**Figure 16:** Cytotoxic effect of purified compound C4 against Vero cell line

The different spectrum of the purified compounds activity against HCT 116 and Vero were showed in Figures 1 –16. The anti-cancerous potential of the purified compounds was showed significant cytotoxicity with the increasing of sample concentration and it was observed in results (Tables3–4). In fact, a good pattern of cytotoxicity activity was observed in HCT 116 and Vero cell line at all tested sample concentrations in 48 hours' treatment it also revealed that increased concentration of drug shown high cytotoxicity over both the HCT 116 and Vero cell lines (Figures12-19). The $IC_{50}$concentration of the selected purified compounds C1, C2, C3, and C4 against HCT 116 were 79.06 µg/ml, 84.13 µg/ml, 94.36 µg/ml and 68.47µg/ml, respectively. Whereas, the $IC_{50}$concentration of the selected purified compounds C1, C2, C3, and C4 against Vero were 130.30µg/ml, 157.36µg/ml, 160.48µg/ml and 255.28µg/ml, respectively. After the anti-cancerous and cytotoxicity evaluation against normal cells of the pure compounds, the compound C4wasselected and subjected to spectral analysis and structural elucidation. In vitro cytotoxicity and/or cell viability assays have some advantages, such as speed, reduced cost, and automation potential, and tests using human cells may be more relevant than certain in vivo animal tests. However, they have some shortcomings because they are not technologically advanced enough to replace animal testing It is important to know how many live cells and/or dead cells are left at the end of the experiment. Currently, a wide range of cytotoxicity and cell viability assays are used in the fields of toxicology and pharmacology. The choice of analysis method is critical to assess the type of interaction.

## CONCLUSION

In vitro cell viability and cytotoxicity assays of cultured cells are widely used in cytotoxicity testing of chemical substances and drug screening. In recent years, the application of these assays has attracted increasing interest. Currently, these assays are also used in oncology research to evaluate the toxicity of compounds and the inhibitory effect of tumor cell growth during drug development. Because they are fast, cheap and do not require the use of animals. In addition, they are useful for testing a large number of samples. Cell viability and cytotoxicity assays are based on various cell functions, such as cell membrane permeability, enzyme activity, cell adhesion, ATP production, coenzyme production and nucleotide uptake activity. The present study indicates that the pure isolated compounds from chloroform extract of *Chrysopogon zizanioides* possess significant cytotoxicity effects on Vero and human colorectal cancer cell lines.

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